

EXHIBIT 18

J. Noah Hagey, Esq.
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March 20, 2020

VIA EMAIL PURSUANT TO SERVICE AGREEMENT

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Re: CCP § 998 Offer to Compromise – *Embry v. B&G Foods North America, Inc., et al.*, Case No. RG20057491 (Alameda County Sup. Ct.)

Dear Counsel:

As you know, we represent Defendant B&G Foods North America, Inc. (“B&G” or “Defendant”) in connection with Plaintiff Kim Embry’s Proposition 65 lawsuit regarding SnackWell’s Devil’s Food Cookie Cakes (the “Cookie Cakes”). We write to provide Defendant’s appended statutory Offer to Compromise pursuant to California Code of Civil Procedure Section 998.¹

As detailed below, Plaintiff’s claims are without merit. Moreover, Plaintiff’s efforts to force Defendant to put false statements on product labels, purportedly on behalf of the State of California, violate Defendant’s rights to free speech and due process under the United States Constitution, giving rise to liability for treble damages and attorneys’ fees under 42 U.S.C. § 1983. We hope Plaintiff will agree to dismiss her infirm claims before Defendant incurs further damages, as well as hundreds of thousands of dollars in anticipated litigation, laboratory, and expert fees that ultimately will be taxed against Plaintiff.

¹ Appended as **Exhibit 1**.

After reviewing this offer with your client, please indicate your client's acceptance by signing the attached offer and filing it with the Court.

A. Plaintiff's Attempts to Compel a False Cancer Warning are Unconstitutional

1. Cookie Cakes are fun to eat and delicious. They do not cause cancer and the State of California should not compel B&G Foods to falsely tell consumers that they are potentially cancerous or anything else that is not true.

2. The Cookie Cakes are reduced fat chocolate cookie cakes with marshmallow and fudge coating. They are free from high fructose corn syrup and partially hydrogenated oils. They are safe and delicious to eat. Like virtually all baked goods, the Cookie Cakes contain trace amounts of acrylamide – a naturally-occurring substance that has been present in human foods since the discovery of fire.

3. Plaintiff's Complaint seeks a court order compelling Defendant to tell consumers that the tiny amounts of acrylamide in the Cookie Cakes are "known to the State of California" to cause cancer.² This compelled statement would be false. There is not a shred of evidence that acrylamide causes cancer in humans, and the State of California has admitted that it does not "know" that acrylamide causes cancer.³ In June 2019, the California Office of Environmental Health Hazard Assessment (OEHHA) adopted a new regulation admitting that acrylamide exposures in coffee "do not pose a significant risk of cancer."⁴

4. Dozens of epidemiological studies show that "there is no consistent or reliable evidence to support a finding that dietary exposure to acrylamide increases the risk of any type of cancer in humans."⁵ The United States Food & Drug Administration has stated that requiring cancer warnings for acrylamide in baked goods like cereals and breads would confuse and mislead consumers.⁶ The National Cancer Institute and the American Cancer Society both recognize that there is no evidence of any association between dietary exposure to acrylamide and cancer.⁷⁸

² Appended as **Exhibit 2**.

³ See **Exhibit 3**, Deposition of Martha Sandy, California Office of Environmental Health Hazard Assessment, May 2, 2007.

⁴ **Exhibit 4**, OEHHA, Final Statement of Reasons, Adoption of New Section 25704 Exposures to Listed Chemicals in Coffee Posing No Significant Risk (June 7, 2019), <https://oehha.ca.gov/media/downloads/crn/fsorcoffee060719.pdf>

⁵ **Exhibit 5**, Declaration of Dr. Loren Lipworth, *California Chamber of Commerce v. Becerra*, Case No. 2:19-cv-02019-KJM-EFB (E.D. Cal. November 8, 2019).

⁶ **Exhibit 6**, Letter from Lester M. Crawford, DVM, Ph.D, Deputy Commissioner, FDA, to Joan E. Denton, M.S., Ph.D, Director, OEHHA (July 13, 2003).

⁷ **Exhibit 7**, NCI, Acrylamide and Cancer Risk (Dec. 5, 2017), <https://www.cancer.gov/about-cancer/causes-prevention/risk/diet/acrylamide-fact-sheet>.

⁸ **Exhibit 8**, American Cancer Society, Acrylamide and Cancer Risk (Feb. 11, 2019), <https://www.cancer.org/cancer/cancer-causes/acrylamide.html>.

5. Plaintiff's attempt to force Defendant to provide a false cancer warning for the Cookie Cakes violates Defendant's right to free speech. Under the First Amendment, laws compelling speech receive strict scrutiny. *Wooley v. Maynard*, 430 U.S. 705, 715-16 (1977). Laws regulating commercial speech generally receive at least intermediate scrutiny, *i.e.*, they are prohibited if they do not directly and materially advance the government's interest, or are more extensive than necessary. *Cent. Hudson Gas & Elec. Corp. v. Pub. Serv. Comm'n*, 447 U.S. 557, 566 (1980).

6. And even laws that require businesses to provide information in connection with commercial transactions are permissible only if the compelled disclosure is of information that is purely factual and uncontroversial, reasonably related to a substantial government purpose, and not unjustified or unduly burdensome. *Nat'l Inst. of Family Life Advocates v. Becerra*, 138 S. Ct. 2361, 2372, 2377; *Zauderer v. Office of Disciplinary Counsel*, 471 U.S. 626, 651 (1985). Because Proposition 65's warning requirement as applied to acrylamide in Cookie Cakes is false, misleading, and factually controversial, it cannot survive any level of constitutional scrutiny. See *Video Software Dealers Ass'n v. Schwarzenegger*, 556 F.3d 950, 967 (9th Cir. 2009) ("[T]he State has no legitimate reason to force retailers to affix false information on their products.").⁹

7. In sum, Plaintiff's effort to coerce Defendant into putting a false cancer warning on the Cookie Cakes and to pay exorbitant "penalties" for the absence of such a warning constitutes impermissible compelled speech under the First Amendment and fails as a matter of law.

B. Plaintiff's Claims are Without Merit Under Proposition 65

8. Plaintiff's claims are also substantively meritless because the trace amounts of acrylamide in the Cookie Cakes comply with Proposition 65, including consent judgment levels ratified by Plaintiff and the California Attorney General.

9. *First*, Plaintiff's claims are without merit because the product named in the Notice of Violation has been discontinued. Defendant no longer sells SnackWell's Devil's Food Fat Free Cookie Cakes, the product named in Plaintiff's Notice of Violation. The Cookie Cakes currently in the market are a different product with a different recipe. Plaintiff cannot obtain injunctive relief or penalties with respect to discontinued products.

10. *Second*, any acrylamide in the Cookie Cakes falls within Proposition 65's statutory exemption for chemicals produced by cooking. Under the exemption, no warning is required for "chemicals in food [] produced by cooking necessary to render the food palatable or to avoid microbiological contamination." Cal. Code Regs. § 25703(b)(1). It is beyond question that cookies and cakes must be baked – otherwise they would be an unpalatable mess of raw flour, sugar, and chocolate. And it is this baking that produces acrylamide, as the FDA has long

⁹ Plaintiff's Complaint also violates Defendant's due process and free speech rights under the void-for-vagueness doctrine. *Grayned v. City of Rockford*, 408 U.S. 104, 108 (1972); *Cal. Teachers Ass'n v. State Bd. of Educ.*, 271 F.3d 1141, 1150 (9th Cir. 2001).

recognized.¹⁰ As such, any acrylamide present in the Cookie Cakes is exempt from warning under Proposition 65.

11. *Third*, even if the cooking exception did not apply, the *de minimis* levels of acrylamide in the cookie cakes are well within the Proposition 65 safe harbor. The Attorney General has repeatedly endorsed acrylamide limits of 275 to 281 parts per billion for snack food products like potato chips, tortilla chips, pretzels, and popcorn, which are consumed with much greater frequency than cookie cakes.¹¹ Subsequent consent judgments have set limits of 350 to 490 ppb or more for other snack foods.¹²

12. Plaintiff herself has expressly admitted in court filings that cookies with up to 280 parts per billion acrylamide comply with Proposition 65.¹³ The acrylamide levels in the Cookie Cakes are well below these Attorney General-approved limits, at between 40 and 75 parts per billion.¹⁴ In other words, even if the cooking exception did not provide a complete defense (which it does), Plaintiff's claims would still fail because the trace levels of acrylamide in the Cookie Cakes comply with the statute.

* * *

In sum, Plaintiff's claims are without merit and violate Defendant's constitutional rights. Defendant intends to vigorously defend Plaintiff's claims in state court and will seek redress for Plaintiff's constitutional violations in its pending Section 1983 lawsuit in federal court. In the state court action, Defendant will incur a myriad of hard costs during the litigation and trial preparation, including discovery and deposition expenses, laboratory and testing expenses, expert retention fees, electronic discovery and database fees and costs, and trial costs. Based on our experience, these costs will total more than \$500,000. Should Plaintiff fail to accept this compromise offer, Defendant will seek to recoup all these costs from Plaintiff pursuant to CCP § 998.

¹⁰ **Exhibit 9**, OEHHA Acrylamide Fact Sheet

¹¹ **Exhibit 10**, Consent Judgment as to Defendant Frito-Lay, Inc., *People v. Snyder's of Hanover Inc., et al.*, Case No. RG09455286 (Alameda Cty. Sup. Ct., Sept. 19, 2011)

¹² **Exhibit 11**, Consent Judgment as to Defendant Inventure Foods, Inc., *Center for Environmental Health v. Snikiddy, LLC, et al.*, Case No. RG16838609 (Alameda Cty. Sup. Ct. Dec. 18, 2018).

¹³ **Exhibit 12**, Proposed Consent Judgment, *Embry v. Nonni's Foods, LLC, et al.*, Case No. HG-17-885297 (Alameda Cty. Sup. Ct. Sept. 25, 2019).

¹⁴ See test results appended as **Exhibit 13**.

We urge Ms. Embry to dismiss her meritless claims and cease violating Defendants' constitutional rights. After you have discussed this offer with your client, please indicate your client's acceptance by signing the attached offer and filing it with the Court.

Very truly yours,

A handwritten signature in blue ink, appearing to be 'J. Noah Hagey', written over the typed name.

J. Noah Hagey

Encl.

EXHIBIT 1

1 J. Noah Hagey, Esq. (SBN: 262331)
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3 Matthew Borden, Esq. (SBN: 214323)
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14 ATTORNEYS FOR DEFENDANT
15 B&G FOODS NORTH AMERICA, INC.

16
17 **SUPERIOR COURT OF THE STATE OF CALIFORNIA**
18 **COUNTY OF ALAMEDA**

19 KIM EMBRY,

20 Plaintiff,

21 v.

22 B&G FOODS NORTH AMERICA, INC.,
23 RALPH'S GROCERY COMPANY, and DOES
24 1-100, inclusive,

25 Defendants.

Case No. RG20057491

**OFFER TO COMPROMISE
PURSUANT TO CODE OF CIVIL
PROCEDURE § 998**

TO PLAINTIFF KIM EMBRY AND HER COUNSEL OF RECORD:

1. Defendant B&G Foods North America, Inc. (“Defendant”), by and through its undersigned and duly authorized counsel of record, and reserving all rights, claims, and defenses, hereby offers to compromise pursuant to California Code of Civil Procedure Section 998 on the following terms (the “Offer”):

a. The above-captioned Complaint against Defendant shall be dismissed with prejudice.

b. Plaintiff shall take nothing by way of the Complaint.

c. Each party shall bear its own fees and costs incurred in this action.

2. The Offer will expire after thirty (30) days. Cal. Code Civ. Proc. § 998(b)(2).

3. The Offer is not an admission of liability or wrongdoing by Defendant as to any of the allegations set forth in the operative complaint. Defendant maintains that Plaintiff’s claims lack merit and that this Offer is an effort by Defendant solely to avoid the further expense and distraction of litigation and to obtain relief from the substantial costs that Defendant will be forced to incur to prove that Plaintiff’s claims are baseless, including without limitation, legal research database costs, discovery and deposition costs, product testing and lab fees, trial logistics and technology costs, and expert witness fees, collectively estimated to exceed \$500,000.

4. Plaintiff should indicate her acceptance of the Offer by signing the acceptance provision below and filing the accepted Offer with the Court. If Plaintiff fails to accept the Offer, Defendant intends to litigate this case to a favorable judgment and seek all recoverable costs and expenses against Plaintiff. *See* Cal. Code Civ. Proc. § 998(c).

Dated: March 20, 2020

BRAUNHAGEY & BORDEN LLP

By: 

J. Noah Hagey
Attorneys for Defendant
B&G Foods North America, Inc.

ACCEPTANCE OF OFFER TO COMPROMISE

Plaintiff Kim Embry accepts the above offer pursuant to Rule 998 of the California Code of Civil Procedure and consents to entry of judgment on the terms above.

Dated: _____

By: _____
Plaintiff Kim Embry

Dated: _____

By: _____
Plaintiff's Attorney of Record

EXHIBIT 2

To: Superior Court of California County of Alameda Page 2 of 9 2020-03-06 17:53:43 (GMT)

16193930154 From: Samantha Dice

FILED BY FAX

ALAMEDA COUNTY

March 06, 2020

CLERK OF
THE SUPERIOR COURT
By Xian-xii Bowie, Deputy

CASE NUMBER:

RG20057491**NICHOLAS & TOMASEVIC, LLP**

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Attorneys for Plaintiff

Kim Embry

SUPERIOR COURT OF THE STATE OF CALIFORNIA**IN AND FOR THE COUNTY OF ALAMEDA**

KIM EMBRY, an individual,

Plaintiff,

v.

B&G FOODS NORTH AMERICA, INC., a
Delaware corporation, RALPHS GROCERY
COMPANY, an Ohio corporation, DOES 1
through 100, inclusive,

Defendants.

Case No.:

**COMPLAINT FOR CIVIL PENALTIES
AND INJUNCTIVE RELIEF**

(Health & Safety Code § 25249.6 et seq.)

I.
INTRODUCTION

1
2 1. This Complaint is a representative action brought by Plaintiff in the public interest of
3 the citizens of the State of California (“the People”). Plaintiff seeks to remedy Defendants’ failure to
4 inform the People of exposure to acrylamide, a known carcinogen. Defendants expose consumers to
5 acrylamide by manufacturing, importing, selling, and/or distributing Snack Well’s Devil’s Food Fat
6 Free Cookie Cakes (“Products”). Defendants know and intend that customers will ingest Products
7 containing acrylamide.

8 2. Under California’s Safe Drinking Water and Toxic Enforcement Act of 1986, California
9 Health and Safety Code, section 25249.6 et seq. (“Proposition 65”), “[n]o person in the course of doing
10 business shall knowingly and intentionally expose any individual to a chemical known to the state to
11 cause cancer or reproductive toxicity without first giving clear and reasonable warning to such
12 individual. . . .” (Health & Safety Code, § 25249.6.)

13 3. California identified and listed acrylamide as a chemical known to cause cancer as early
14 as January 1, 1990, and as a chemical known to cause developmental/reproductive toxicity in February
15 of 2011.

16 4. Defendants failed to sufficiently warn consumers and individuals in California about
17 potential exposure to acrylamide in connection with Defendants’ manufacture, import, sale, or
18 distribution of Products. This is a violation of Proposition 65.

19 5. Plaintiff seeks injunctive relief compelling Defendants to sufficiently warn consumers
20 in California before exposing them to acrylamide in Products. (Health & Safety Code, § 25249.7(a).)
21 Plaintiff also seeks civil penalties against Defendants for their violations of Proposition 65 along with
22 attorney’s fees and costs. (Health & Safety Code, § 25249.7(b).)

II.
PARTIES

23
24
25 6. Plaintiff KIM EMBRY (“Embry”) is a citizen of the State of California dedicated to
26 protecting the health of California citizens through the elimination or reduction of toxic exposure from
27 consumer products. She brings this action in the public interest pursuant to Health and Safety Code,
28 section 25249.7.

7. Defendant B&G FOODS NORTH AMERICA, INC. (“B&G”), is a corporation organized and existing under the laws of Delaware. B&G is registered to do business in California, and does business in the County of Alameda, within the meaning of Health and Safety Code, section 25249.11. B&G manufactures, imports, sells, or distributes the Products in California and Alameda County.

8. Defendant RALPHS GROCERY COMPANY (“Ralphs”), is a corporation organized and existing under the laws of Ohio. Ralphs is registered to do business in California, and does business in the County of Alameda, within the meaning of Health and Safety Code, section 25249.11. Ralphs manufactures, imports, sells, or distributes the Products in California and Alameda County.

9. Plaintiff does not know the true names and/or capacities, whether individual, partners, or corporate, of the defendants sued herein as DOES 1 through 100, inclusive, and for that reason sues said defendants under fictitious names. Plaintiff will seek leave to amend this Complaint when the true names and capacities of these defendants have been ascertained. Plaintiff is informed and believes and thereon alleges that these defendants are responsible in whole or in part for Plaintiffs' alleged damages.

III. VENUE AND JURISDICTION

10. California Constitution Article VI, Section 10 grants the Superior Court original jurisdiction in all cases except those given by statute to other trial courts. The Health and Safety Code statute upon which this action is based does not give jurisdiction to any other court. As such, this Court has jurisdiction.

11. Venue is proper in Alameda County Superior Court pursuant to Code of Civil Procedure, sections 394, 395, and 395.5. Wrongful conduct occurred and continues to occur in this County. Defendants conducted and continue to conduct business in this County as it relates to Products.

12. Defendants have sufficient minimum contacts in the State of California or otherwise purposefully avails itself of the California market. Exercising jurisdiction over Defendants would be consistent with traditional notions of fair play and substantial justice.

**IV.
CAUSES OF ACTION**

**FIRST CAUSE OF ACTION
(Violation of Proposition 65 – Against all Defendants)**

13. Plaintiff incorporates by reference each and every allegation contained above.

14. Proposition 65 mandates that citizens be informed about exposures to chemicals that cause cancer, birth defects, and other reproductive harm.

15. Defendants manufactured, imported, sold, and/or distributed Products containing acrylamide in violation of Health and Safety Code, section 25249.6 et seq. Plaintiff is informed and believes such violations have continued after receipt of the Notice (defined *infra*) and will continue to occur into the future.

16. In manufacturing, importing, selling, and/or distributing Products, Defendants failed to provide a clear and reasonable warning to consumers and individuals in California who may be exposed to acrylamide through reasonably foreseeable use of the Products.

17. Products expose individuals to acrylamide through direct ingestion. This exposure is a natural and foreseeable consequence of Defendants placing Products into the stream of commerce. As such, Defendants intend that consumers will ingest Products, exposing them to acrylamide.

18. Defendants knew or should have known that the Products contained acrylamide and exposed individuals to acrylamide in the ways provided above. The Notice informed Defendants of the presence of acrylamide in the Products. Likewise, media coverage concerning acrylamide and related chemicals in consumer products provided constructive notice to Defendants.

19. Defendants' action in this regard were deliberate and not accidental.

20. More than sixty days prior to naming each defendant in this lawsuit, Plaintiff issued a 60-Day Notice of Violation ("Notice") as required by and in compliance with Proposition 65. Plaintiff provided the Notice to the various required public enforcement agencies along with a certificate of merit. The Notice alleged that Defendants violated Proposition 65 by failing to sufficiently warn consumers in California of the health hazards associated with exposures to acrylamide contained in the Products.

21. The appropriate public enforcement agencies provided with the Notice failed to commence and diligently prosecute a cause of action against Defendants.

22. Individuals exposed to acrylamides contained in the Products through direct ingestion resulting from reasonably foreseeable use of the Products have suffered and continue to suffer irreparable harm. There is no other plain, speedy, or adequate remedy at law.

23. Defendants are liable for a maximum civil penalty of \$2,500 per day for each violation of Proposition 65 pursuant to Health and Safety Code, section 252497(b). Injunctive relief is also appropriate pursuant to Health and Safety Code, section 25249.7(a).

PRAYER FOR RELIEF

Wherefore, Plaintiff prays for judgment against Defendants as follows:

1. Civil penalties in the amount of \$2,500 per day for each violation;
2. A preliminary and permanent injunction against Defendants from manufacturing, importing, selling, and/or distributing Products in California without providing a clear and reasonable warning as required by Proposition 65 and related Regulations;
3. Reasonable attorney's fees and costs of suit; and
4. Such other and further relief as may be just and proper.

Respectfully submitted:

Dated: March 6, 2020

GLICK LAW GROUP, PC

By:



Noam Glick
Attorney for Plaintiff

EXHIBIT 3

EXHIBIT 4

EXHIBIT 5

TRENTON H. NORRIS (CA Bar No. 164781)
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UNITED STATES DISTRICT COURT
EASTERN DISTRICT OF CALIFORNIA

CALIFORNIA CHAMBER OF
COMMERCE,

Plaintiff,

v.

XAVIER BECERRA, IN HIS OFFICIAL
CAPACITY AS ATTORNEY GENERAL
OF THE STATE OF CALIFORNIA,

Defendant.

Civil Action No. 2:19-cv-02019-KJM-EFB

**DECLARATION OF DR. LOREN
LIPWORTH IN SUPPORT OF
PLAINTIFF'S MOTION FOR A
PRELIMINARY INJUNCTION**

DECLARATION OF DR. LOREN LIPWORTH

I, Dr. Loren Lipworth, declare under penalty of perjury as follows:

1. I submit this Declaration in support of Plaintiff's Motion for Preliminary Injunction. I have personal knowledge of the matters stated herein, and if called to do so, I could and would competently testify to each of the facts and opinions set forth below.

I. REPORT OUTLINE

2. In this report, I begin by describing my background and qualifications as a specialist in the field of epidemiology. I then lay out important concepts in epidemiology that should be considered in the evaluation of epidemiologic research, including the strength and limitations of different study designs and bias. Next, I detail the methods of the literature review that I conducted according to standard methodology that is widely accepted in the field of epidemiology. I then describe the key findings for the objective of the literature review and conclude the report by stating my opinions in this matter.

II. SCOPE OF OPINION

3. All the opinions stated in this report are stated to a reasonable degree of scientific certainty. I reserve the right to supplement or amend my opinions based upon any new information or literature that subsequently becomes available. In addition, I reserve the right to discuss general concepts within the field of epidemiology to provide context for any of the opinions discussed in this report.

4. All of the opinions stated in this report are my own and do not represent those of Vanderbilt University.

5. As set forth in more detail hereafter, it is my opinion that there is no reliable epidemiologic evidence that dietary exposure to acrylamide is causally associated with the development of cancer in humans.

III. QUALIFICATIONS AND BACKGROUND

6. I am qualified as a specialist in the field of epidemiology. Attached as **Exhibit A** is a true and correct copy of my curriculum vitae, which describes my education, training, professional experience and publications.

1 7. I received a Bachelor of Science degree with Honors in Neuroscience from Brown
2 University in 1991. I received a Doctor of Science degree in Epidemiology in 1996 from the Harvard
3 School of Public Health.

4 8. Since 1998, I have served on the faculty of Vanderbilt University School of Medicine
5 in Nashville, Tennessee, first as Assistant Professor, Department of Preventive Medicine (1998-
6 2011), then as Associate Professor (2011-2018) and currently as Professor (2018-present), Division
7 of Epidemiology, Department of Medicine.

8 9. I am a member of the Vanderbilt Epidemiology Center (VEC), the Vanderbilt-Ingram
9 Cancer Center (VICC) Cancer Epidemiology Research Program, the Vanderbilt Center for Kidney
10 Disease (VCKD), and the Vanderbilt Translational and Clinical Cardiovascular Research Center (V-
11 TRACC).

12 10. I routinely provide editorial review to numerous peer-reviewed scientific journals,
13 including Journal of the National Cancer Institute, American Journal of Epidemiology, International
14 Journal of Cancer, Cancer Causes and Control, and Nutrition and Cancer. I am a past Associate
15 Editor of the American Journal of Epidemiology (2010-2014).

16 11. As a member of the Vanderbilt University School of Medicine faculty, I teach
17 graduate students and medical students, and I serve as a research mentor for Epidemiology PhD
18 candidates and post-doctoral fellows, as well as medical residents and fellows. In particular, I have
19 taught courses in Cancer Epidemiology Methods to medical students, and I currently teach a graduate
20 level Epidemiology course entitled "Current Topics in Research." In addition, I teach annual lectures
21 on "Obesity, Energy Balance, and Cancer" for the Vanderbilt Post-doctoral Training Program in
22 Molecular and Genetic Epidemiology (2013-present), and on "Epidemiology for the Biochemical and
23 Molecular Toxicologist" for the Biochemistry PhD and Post-doctoral Program (2015-present).

24 12. I have conducted extensive epidemiologic research. My research has been funded by
25 the National Cancer Institute, the National Heart Lung and Blood Institute, and the National Institute
26 of Diabetes and Digestive and Kidney Diseases, among others. I have published over 180 articles in
27 the peer-reviewed medical literature.

28 13. I authored the following peer-reviewed qualitative review regarding cancer risk in

1 relation to dietary acrylamide intake, which was published in the European Journal of Cancer
2 Prevention: Lipworth L, Sonderman JS, Tarone RE, McLaughlin JK. Review of epidemiologic
3 studies of dietary acrylamide intake and the risk of cancer. Eur J Cancer Prev 2012;21:375-386
4 (“Lipworth et al. 2012”). A true and correct copy of Lipworth et al. 2012 is attached hereto as

5 **Exhibit B.**

6 14. I have not testified as an expert at trial or by deposition in the previous four years.

7 15. I was retained by outside counsel for Plaintiff to provide an independent expert
8 opinion on the epidemiological data regarding dietary acrylamide and cancer. I am being
9 compensated for my time at a rate of \$500 per hour.

10 **IV. EPIDEMIOLOGICAL STUDIES ON DIETARY ACRYLAMIDE AND CANCER**

11 **A. General Principles of Epidemiology**

12 16. Epidemiology provides the basis for the evaluation of whether a particular exposure
13 may be associated with the risk of disease in human populations. Its approach is population based, as
14 compared to a clinical or individual perspective. The existence of an association in an epidemiologic
15 study does not mean that there is a cause and effect relationship. Inferences about causation require
16 additional evaluation and judgment. Conversely, the absence of an association in a well-conducted
17 epidemiologic study is evidence that there is not a cause and effect relationship, and this inference is
18 strengthened if there is consistency in results across multiple studies.

19 17. Conclusive empirical evidence regarding disease causation in humans can, in theory,
20 only come from double-blind randomized trials in humans. Randomization ensures equal distribution
21 of both known and unsuspected confounding factors, and double-blind designs minimize the potential
22 for several types of bias. However, experimental studies of disease causation often cannot be
23 undertaken in humans, for practical or ethical reasons, except when there is evidence that a particular
24 factor may actually protect from disease (e.g., clinical drug trials). Even under these conditions, trials
25 aiming at disease prevention in humans often are impractical or extremely complex.

26 18. In the absence of human experimental evidence, disease causation in humans can best
27 be assessed through observational or non-experimental studies, and the most effective among these
28 are analytic epidemiologic investigations. Cohort and case-control studies are examples of analytic

1 epidemiologic investigations.

2 19. A cohort study (or follow-up study) identifies populations of individuals exposed or
3 not exposed to a particular factor and follows them forward in time. The occurrence of disease in the
4 exposed group is then compared with the occurrence in the nonexposed group, and a relative risk
5 (RR) among exposed as compared to nonexposed is calculated as the ratio of these two occurrence or
6 incidence measures. (For example, a RR of 1.0 -- often called the null value -- means there is no
7 association between the exposure and the disease, since those exposed are just as likely to get the
8 disease as those who are not. And a RR of 2.0 means that the exposure is associated with a doubling
9 of the risk of disease occurrence, and similarly a RR of 0.5 means that the exposure reduces the risk
10 of the disease by half). Cohort studies can be either prospective, relying on current exposure
11 information and identifying new cases of disease as they occur over time, or retrospective, based on
12 preexisting records of exposure and disease occurrence.

13 20. While cohort studies follow individuals from exposure to disease development, case-
14 control studies begin with a group of subjects who have the disease (cases) and a group of subjects
15 who do not have the disease (controls) and then seek to ascertain previous exposure experience. The
16 proportion of cases with a particular exposure is compared with that of the controls in order to
17 determine whether there is an association between the exposure and the disease. The primary
18 challenge in case-control studies is the unbiased selection of cases and appropriate controls from the
19 same source, uninfluenced by their exposure status. Information on exposure in case-control studies
20 is frequently obtained by use of a questionnaire. The potential for information bias concerning
21 exposure to suspected agent(s) is greater in case-control investigations, since the existence of a
22 serious disease can influence recollection and reporting (recall bias) and can affect certain
23 biochemical and immunological variables.

24 21. Well-conducted case-control and/or cohort studies are needed before scientific
25 judgments can be made about whether an exposure to a substance is associated with a disease. By
26 contrast, anecdotal evidence (case reports, case series or other anecdotal data regarding the
27 occurrence of a disease in an individual or group of individuals with an exposure), cannot provide
28 evidence for an association due to the absence of an appropriate comparison group. Both case-

1 control and cohort studies obtain data on suspected risk factors and disease occurrence at the
2 individual level and, by comparing groups of subjects with the exposure of interest and without the
3 exposure of interest, while controlling for other relevant variables that could independently influence
4 the risk of disease, epidemiologists determine whether a statistical association exists between the
5 studied exposure and disease and estimate its magnitude.

6 22. Results from individual studies such as cohort or case-control studies may be
7 combined in a meta-analysis. A meta-analysis is a systematic statistical approach that combines
8 results from individual studies in order to generate a single comprehensive summary measure. A
9 primary goal of meta-analysis is to provide a more precise estimate of the direction and magnitude of
10 effect of an exposure-outcome association. By combining data from many studies into a single
11 summary measure, a meta-analysis can overcome issues in interpretation that may arise when
12 summarizing individual studies that may have small sample sizes or multiple small subgroup
13 comparisons.

14 **B. Interpretation of Epidemiologic Data**

15 23. Because of the absence of randomization in case-control and cohort studies, it is
16 paramount that other potential explanations for any observed exposure-disease association, including
17 the roles of bias (systematic error), confounding (a mixing of an extraneous variable in an exposure-
18 disease association) and chance (statistical significance), be confidently excluded in individual
19 studies before a reported exposure-disease association can be assessed for cause and effect.

20 24. Bias, or systematic error, generally results from flaws in observational study designs
21 and data collection and cannot be corrected at the data analysis stage. There are several types of bias
22 which may be present in any given study, including selection and information bias. Selection bias
23 involves systematic differences in the exposure under study between those selected and those not
24 selected for inclusion in the study. For example, a case-control study that compares oral contraceptive
25 use among women with breast cancer to that among hospitalized women with coronary heart disease
26 may show more estrogen use among the cases (breast cancer) and less among the controls (heart
27 disease) because of a possible protective effect in the latter group. Information bias involves
28 systematic differences in measuring the exposure of interest between the compared groups and

1 includes recall and interview bias. For example, the finding of greater use of oral contraceptives
2 among women with breast cancer than among “healthy” women chosen from a general population
3 may reflect different degrees of recall about past use of oral contraceptives in the two groups

4 25. Another critical factor to be considered when assessing causation is the potential effect
5 of confounding. Confounding refers to the effect of an extraneous variable, related to both exposure
6 and disease, that may partially or completely account for an apparent association between a study
7 exposure and disease. Common confounding variables include age, sex, education, cigarette smoking,
8 alcohol consumption, obesity, or family history of disease. Only when potential confounding
9 variables have been identified and measured can confounding be evaluated in the analysis by
10 stratification of study subjects according to those variables. A confounding variable that is not
11 accurately measured can lead to residual confounding, meaning confounding that is unaccounted for
12 even after statistical adjustment.

13 26. As explained above, case-control and cohort studies use the RR to measure the
14 strength of the association between exposure and disease in the study population. The RR, like an
15 average, is a summary measure of the study data that is intended to be representative of the entire
16 population, but of course does not include every person in the population. Accordingly, statistics
17 play an important role in evaluating epidemiological associations.

18 27. Two statistical tools used in epidemiology for assessing the role of chance are the p-
19 value and the confidence interval (CI). The p-value is the probability of observing, by chance alone, a
20 difference in disease occurrence between the exposed and unexposed as large as or larger than what
21 was actually observed, assuming that the exposure in fact has no effect on disease occurrence and that
22 differences in disease rates are due solely to chance. The smaller the p-value, the less consistent are
23 the data with the null hypothesis of no association. For example, a p-value of 0.005 means that the
24 probability of obtaining by chance alone an exposure effect as large as or more extreme than what
25 was observed is only 1 in 200. Based on this result, one can conclude either that a rare "chance event"
26 has occurred or, more likely, chance can be ruled out as an explanation for the study results. P-values
27 smaller than 0.05, a threshold value often referred to as the alpha or nominal level, are generally
28 referred to as "statistically significant," although they are frequently overemphasized in the

1 interpretation of epidemiologic data. A p-value of 0.05 does not completely rule out chance as an
2 explanation for the results; rather, it means that chance could explain the observed risk estimate only
3 one out of 20 times. The p-value gives no indication of the observed magnitude of the effect of the
4 exposure. Therefore, it is important to present a quantitative estimate of the effect of exposure, along
5 with a measure of the uncertainty of the estimate (the standard error), often presented as a CI.

6 28. The CI provides a range of values containing the true RR with a desired degree of
7 confidence, usually 95%. The "true" RR is the risk that would be observed based on an infinite
8 amount of data, that is, if study sampling variability could be eliminated and the entire population
9 could be studied. For example, if an epidemiologist observes a RR of 1.14 and the 95% CI is 0.93-
10 1.38, it means that the epidemiologist is 95% confident that the true RR falls between 0.93 and 1.38.
11 This implies that a value of 1.0 for the relative risk, and thus the null hypothesis of no exposure
12 effect, is consistent with the data, because 1.0 lies within the 95% CI. Said differently, there is only a
13 5% likelihood that the average of the entire population would be below 0.93 or above 1.38. When 1.0
14 lies within the 95% CI, it is common to say that the study shows no statistically significant
15 association between the exposure and disease.

16 29. Once bias and confounding can be reasonably excluded as likely explanations for an
17 observed association between an exposure and disease, and the results are reasonably inconsistent
18 with chance as an explanation, then the likelihood of a causal association can be assessed using an
19 accepted set of criteria or principles first put forward by Sir Austin Bradford Hill and used in 1964 by
20 the US Surgeon General to link cigarette smoking and lung cancer. These criteria include:

- 21 a) *Strength of the association.* In general, the higher the risk estimate, the less likely the
22 finding may be accounted for by bias, uncontrolled confounding, or chance;
23 b) *Consistency.* The plausibility of a causal association is increased greatly if similar results
24 are reported by various investigators using different study designs in different populations.
25 This criterion is the cornerstone for an overall assessment of evidence, for without this
26 consistency, we, as epidemiologists, cannot rule out that an observed association may be due
27 to methodological flaws of an individual study or to chance;
28 c) *Dose-response effect.* If the risk of disease increases with increasing exposure, a causal

1 interpretation of the association is more plausible;

2 d) *Time sequence*. The exposure must precede the development of the disease in order for it
3 to have caused the disease; and

4 e) *Biologic plausibility*. Does the exposure-disease association make biologic sense given
5 what is known about the natural history of the disease? Is the association consistent with
6 experimental evidence?

7 30. Given the multitude of methodologic challenges that epidemiologic studies confront,
8 the variability of the conditions under which they are undertaken, and the abundance of potential
9 sources of confounding and bias, it is remarkable that their results are often as consistent as they are.
10 It should be stressed that in epidemiology, more than in any other field of biomedical research, it is
11 the collective evidence that is important, rather than the results of a particular study, however large
12 and well done it may be.

13 C. Literature Review Methodology

14 31. A structured literature review of the peer-reviewed, published literature in PubMed
15 (www.pubmed.gov) was conducted to identify relevant epidemiologic studies on dietary acrylamide
16 intake and cancer outcomes using the following search string: (('acrylamide') AND 'cancer') AND
17 ('epidemiology' OR 'case-control' OR 'cohort')). This search string is identical to that used in my
18 previous comprehensive review of the literature (Lipworth et al., 2012). The PubMed search was
19 conducted on July 8, 2019. The same search was conducted again on October 11, 2019 and confirmed
20 that no subsequent articles had been published in the interim.

21 32. The literature from 2011-2019 was examined to capture studies published subsequent
22 to my previous review (Lipworth et al. 2012).

23 33. Studies were included in my review if they met the following criteria:

- 24 a. Study Design: Observational studies (cohort, case-control, case-cohort, nested
25 case-control), systematic reviews, and/or meta-analyses of observational studies
- 26 b. Population: Any human population worldwide
- 27 c. Exposure: Dietary acrylamide intake
- 28 d. Outcome: Any cancer outcome.

34. Studies were excluded from my review if they exhibited any of the criteria below:

- a. Study design: Case reports, opinions, narrative reviews, editorials
- b. Population: Not human
- c. Exposure: Only single acrylamide-containing foods; no exposure of interest
- d. Outcome: No cancer outcome
- e. Other: Study not in English language; included in Lipworth et al. 2012 review.

35. A total of 66 articles were identified using the search string for the time period January 1, 2011 to October 11, 2019. The reference lists from relevant systematic reviews and meta-analyses identified in the search were also considered for full text review.

36. The titles and abstracts of the 66 articles were systematically reviewed, and 34 were considered eligible for full text review. Papers were found ineligible for the following reasons: no cancer outcome of interest (fourteen papers), no dietary acrylamide exposure of interest (nine papers), single acrylamide-containing foods (two papers), not human (one paper), previously included in the Lipworth et al. 2012 review (five papers), and not in English language (one paper).

37. No additional papers were identified from reference lists of systematic review or meta-analyses identified in the search. An additional PubMed search conducted on October 11, 2019 using the following search string - ((acrylamide) AND cancer) AND (RCT or randomized controlled trial*) - did not identify any randomized controlled trials of dietary acrylamide intake and cancer.

38. After full text review of the 34 eligible articles, 10 papers were found to be ineligible and were excluded for the following reasons: no dietary acrylamide exposure of interest (two papers), no incident cancer outcome of interest (three papers), and no new data (five papers).

39. Thus, in total, I reviewed 24 articles from 2011-2019 (true and correct copies of which are attached as **Exhibit C**), as well as almost 30 articles published prior to 2011 that are summarized in Lipworth et al. 2012, in forming my opinions in this case.

D. Results

40. My 2012 review (Lipworth et al. 2012) of the almost 30 relevant epidemiologic studies published at that time concluded that there was no consistent evidence that dietary acrylamide exposure increases the risk of any type of cancer in humans, either overall or among non-smokers.

1 The papers published since 2011 and summarized below reinforce, and strengthen, my conclusion
2 that there is no consistent evidence that dietary acrylamide exposure increases the risk of any type of
3 cancer in humans.

4 41. The 24 papers published since 2011 and included in this review include 22 papers that
5 report data from individual studies on the potential association between dietary acrylamide and
6 cancer, one updated meta-analysis (Pelucchi et al. 2015) and one pooled analysis of endometrial
7 cancer in four previously published cohorts (Je et al. 2015).

8 42. Of the 22 individual study reports, 20 were prospective designs (10 full-cohort
9 analyses, 10 case-cohort or nested case-control studies), and two were retrospective case-control
10 studies.

11 43. Seven of the 22 individual study reports derived from the Netherlands Cohort Study
12 on Diet and Cancer (NLCS) and six from the European Prospective Investigation into Cancer and
13 Nutrition (EPIC) study in Europe; the remaining reports were from the Japan Public Health Center-
14 based Prospective Study (JPHC) (three reports), the Health Professionals Follow-up Study
15 (HPFS)/Nurses' Health Study (NHS) in the United States (two reports), or other study populations in
16 the United States or Europe (four reports).

17 44. In most studies, dietary acrylamide intake was assessed using a single or, in a few
18 studies, repeated food frequency questionnaire, combined with computation of intake using national
19 and international databases of the average acrylamide content in individual foods. Food frequency
20 questionnaires include a list of foods (or groups of foods) and beverages with a response section for
21 respondents to indicate how often they eat or drink each food or beverage. Some food frequency
22 questionnaires include portion sizes (semi-quantitative) while others do not (non-quantitative).
23 Nutrient intake is calculated by multiplying the frequency of consumption of each food by the
24 amount of nutrient in a serving of that food using reference databases.

25 45. The advantages of the food frequency questionnaire are that it is generally
26 representative of foods eaten habitually, and measurement of foods with high day-to-day variability
27 in consumption amounts can be captured accurately. Disadvantages include that it is a retrospective
28 method that relies on respondent memory and that the list of foods included is not all-encompassing,

especially for foods specific to certain ethnic groups. But importantly, using a food frequency questionnaire along with the average values reported in national databases reliably ranks individuals into high or low acrylamide intake categories, even if estimated absolute amounts of acrylamide intake are less precise and variations in acrylamide concentrations within individual foods exist by brand or method of preparation.

46. In the studies I reviewed, RRs were presented for a comparison of the highest category of acrylamide intake versus the lowest (a categorical analysis), and, in some studies, RRs were also presented for a continuous measure of intake, represented as an absolute increase of 10 µg/day in acrylamide intake. The RR from the continuous analysis represents the relative increase in risk for every additional 10 µg/day of acrylamide intake.

47. Tobacco smoke is an important non-dietary source of acrylamide exposure (Schettgen et al., 2004; Hagmar et al., 2005; Bjellaas et al., 2007; Vesper et al. 2007); thus, most epidemiologic studies have evaluated the relation between dietary acrylamide intake and the risk of cancer restricted to a subgroup of never smokers.

48. In many populations, coffee is one of the major food sources of acrylamide. Some studies have conducted secondary analyses with statistical adjustment for coffee consumption, while a few studies have examined the relation between dietary acrylamide intake and risk of cancer across different categories of coffee consumption. These additional coffee-specific adjustments and analyses did not materially change the results and therefore they are not presented in the individual study summaries below.

49. Three studies (Xie et al. 2013; Obon-Santacana et al. 2016a, 2016b) evaluated biomarkers of acrylamide exposure (e.g. acrylamide-hemoglobin (Hb) and glycidamide-Hb adducts). Among non-smokers, adduct levels are assumed to primarily represent acrylamide exposure from diet, so these studies were included in our review although they did not directly consider dietary acrylamide intake.

50. The results of the reviewed studies are presented below, by cancer site.

a. **Esophageal Cancer**

51. Two cohort studies (Lugjan-Barroso et al. 2014; Liu et al. 2019), as well as the 2015

meta-analysis (Pelucchi et al. 2015), have examined dietary acrylamide intake in relation to esophageal cancer.

52. The non-statistically significant summary RR for esophageal cancer from the 2015 meta-analysis, based on 4 studies and 1,546 cases, was 1.14 (95% CI 0.93-1.38).

53. The study from the EPIC cohort (Lugian-Barroso et al. 2014) was included in the meta-analysis (Pelucchi et al. 2015) and included 341 esophageal cancer cases observed after more than 5 million person-years of follow-up. The relative risk for the highest level of acrylamide intake was not statistically significantly elevated either overall (RR=1.41; 95% CI 0.86-2.71), or by esophageal cancer subtype (squamous cell carcinoma and adenocarcinoma) or among never smokers and those who hadn't smoked in more than 20 years.

54. The most recent study of acrylamide intake and esophageal cancer was conducted among over 140,000 men and women in the JPHC with approximately 15.5 years of follow-up (Liu et al. 2019). The mean levels of dietary acrylamide intake in the Japanese population were 6.8 µg/day overall and 12.7 µg/day for the highest quartile, considerably lower than in other populations studied in the US and Europe. The results also demonstrate no association, with a non-statistically significant RR of 0.84 (95% CI 0.59-1.19) for the highest level of intake.

55. In my opinion, the available epidemiologic evidence does not support an association between dietary acrylamide intake and increased risk of esophageal cancer.

b. Stomach Cancer

56. One cohort study (Liu et al. 2019), as well as the 2015 meta-analysis (Pelucchi et al. 2015), has examined the association of dietary acrylamide intake and stomach cancer.

57. In the Pelucchi et al. (2015) meta-analysis, based on 787 cases of stomach cancer observed in 2 European studies, both published prior to the Lipworth et al. 2012 review, the summary RR was close to the null value of 1.0 (meaning no association) for both the highest vs. lowest category of dietary acrylamide intake (RR=1.03; 95% CI 0.79-1.03) and for a 10µg/day increase in acrylamide intake (RR=1.01; 95% CI 0.96-1.07), and neither result was statistically significant.

58. The JPHC study (Liu et al. 2019) also examined acrylamide intake in relation to gastric cancer. Based on almost three times as many cases as included in the 2015 meta-analysis

(N=2,218), the RR was 0.90 (95% CI 0.79-1.04) for the highest level of intake, indicating a negative association and one that is not statistically significant.

59. Thus, in my opinion, the collective epidemiologic evidence does not support an association between dietary acrylamide intake and stomach cancer.

c. **Colorectal Cancer**

60. Two epidemiologic cohort studies (Hogervorst et al. 2014; Liu et al. 2019), as well as a meta-analysis (Pelucchi et al. 2015), have examined the association of dietary acrylamide intake and colorectal cancer.

61. In the meta-analysis conducted by Pelucchi et al. (2015), which included the Hogervorst et al. (2014) study, 6,794 cases were identified in six studies. The summary RR for colorectal cancer was not statistically significant and close to or below the null value for the highest vs. lowest category of dietary acrylamide intake (RR=0.94; 95% CI 0.85-1.04) and for a 10 µg/day increase in acrylamide intake (RR=1.00; 95% CI 0.98-1.01).

62. The case-cohort analysis (Hogervorst et al. 2014) conducted in the NLCS cohort was based on 623 colorectal cancer cases identified over 7.3 years of follow-up. Among men (341 cases), the RRs for both the highest vs. lowest category of dietary acrylamide intake (RR=1.17; 95% CI: 0.82-1.66) and for a 10 µg/day increase in acrylamide intake (RR=1.03; 95% CI: 0.94-1.14) were not statistically significant. Among women (282 cases), the RR for the highest vs. lowest category of dietary acrylamide intake (RR=0.76; 95% CI: 0.52-1.11) and for a 10 µg/day increase in acrylamide intake (RR=0.95; 95% CI: 0.85-1.07) were both reduced, but neither result was statistically significant.

63. The recent JPHC cohort study (Liu et al. 2019) also evaluated acrylamide intake with respect to colorectal cancer. Over a follow-up period of 15.3 years, 2,470 cases of colorectal cancers were identified within the cohort. The RR was not increased for the highest level of dietary acrylamide intake (RR=0.93; 95% CI: 0.79-1.08), consistent with the findings of the meta-analysis (Pelucchi et al. 2015).

64. In my opinion, the available epidemiologic evidence does not support an association between dietary acrylamide intake and risk of colorectal cancer.

d. **Pancreatic Cancer**

65. Two epidemiologic studies, one cohort (Obon-Santacana et al. 2013) and one pooled case-control (Pelucchi et al. 2017), as well as the 2015 meta-analysis (Pelucchi et al. 2015), have examined dietary acrylamide intake in relation to pancreatic cancer risk.

66. The 2015 meta-analysis (Pelucchi et al. 2015) reported, based on 4 studies with 1,732 pancreatic cancer cases, a non-statistically significant summary RR below 1.0 (RR=0.93; 95% CI 0.76-1.12 for the highest vs. lowest level of intake and RR=0.99; 95% CI 0.95-1.03 for a 10 µg/day increase in acrylamide intake).

67. In the European EPIC cohort (Obon-Santacana et al. 2013), the results of which were included in the 2015 meta-analysis, 865 cases of pancreatic cancer were observed after more than 5 million person-years of follow-up, yielding a RR of 0.77 (95% CI 0.58-1.04) for the highest quintile of acrylamide intake compared to the lowest, with corresponding RRs of 0.67 (95% CI 0.45-1.00) among women and 0.99 (95% CI 0.60-1.61) among men. The RR for the highest quintile of acrylamide intake among never smokers was also non-statistically significant and below 1.0 (RR=0.86; 95% CI 0.52-1.41).

68. The International Pancreatic Cancer Case–Control Consortium (PanC4) is a consortium of six case-control studies conducted at four sites in the US, one in Italy and one in Australia (Pelucchi et al. 2017). In a combined analysis of 1,975 cases and 4,239 controls (more than the total number of cases included in the 2015 meta-analysis), the RR was 0.92 (95% CI 0.66-1.28) for the highest versus lowest acrylamide intake. Among never smokers, the pooled RR was 1.08 and not statistically significant (95% CI 0.79-1.47). Three of the RR estimates from the individual case-control studies in the pooled analysis were below 1.0, one significantly so (MD Anderson, USA), and none was statistically significantly elevated, consistent with the absence of an association with pancreatic cancer in all 4 earlier studies included in the 2015 meta-analysis.

69. In my opinion, the available epidemiologic evidence does not support an association between dietary acrylamide intake and increased risk of pancreatic cancer.

e. **Kidney Cancer**

70. Two studies conducted since 2011 (Graff et al. 2018; McCullough et al. 2019), as well

1 as the meta-analysis by Pelucchi et al. (2015), have examined the association of dietary acrylamide
2 intake and kidney cancer.

3 71. In the Pelucchi et al. (2015) meta-analysis, based on 1,802 cases of kidney cancer
4 observed in 5 studies, all of which were published prior to the Lipworth et al. 2012 review, the
5 summary RR for the highest vs. lowest category of dietary acrylamide intake was 1.20 (95% CI 1.00-
6 1.45; p for heterogeneity=0.16) using a fixed effects model. The fixed effects model was selected
7 based on an *a priori* decision by the authors to use fixed effects models when the p-value for
8 heterogeneity was > 0.10 and random effects models when the p-value for heterogeneity
9 was ≤ 0.10 . This result is at the borderline of statistical significance with a lower confidence limit of
10 1.00. In contrast, the p-value for heterogeneity was statistically significant (p=0.03) for the
11 continuous measure of dietary acrylamide intake, and thus the RR for a 10 μ g/day increase in
12 acrylamide intake was reported from a random effects model as 1.02 (95% CI 0.97-1.08). This result
13 is not statistically significant. When comparing highest vs. lowest level of acrylamide intake, the
14 summary RR for never or former smokers was 1.10 (95% CI 0.63-1.92) and for current smokers was
15 1.28 (95% CI 0.78-2.11), both not statistically significant.

16 72. The CPS-II Nutrition Cohort study was established by the American Cancer Society in
17 1999. A total of 412 renal cell cancer cases were observed among 102,154 individuals with over 14
18 years of follow up (McCullough et al. 2019). No statistically significant increase in kidney cancer
19 risk was observed among either men (0.97; 95% CI 0.68-1.41) or women (RR=1.26; 95% CI 0.83-
20 1.93) with the highest level of dietary acrylamide intake, nor was there an increased risk among never
21 smokers (RR=1.07; 95% CI 0.68-1.69).

22 73. Kidney cancer was also evaluated in two large prospective cohorts in the US, the
23 HPFS and the NHS, with a combined 629 cases and median follow-up of 27.2 and 33.9 years,
24 respectively (Graff et al. 2018). Among men in the HPFS with the highest level of acrylamide intake,
25 the RR was 1.09 (95% CI 0.77-1.55), and the corresponding estimate among women in the NHS was
26 0.85 (95% CI 0.61-1.17); the summary RR was 0.95 (95% CI 0.74-1.22). None of the results was
27 statistically significant. Likewise, no statistically significant increased RR for the highest vs. lowest
28 quartile of acrylamide intake was observed when analyses were restricted to non-smoking men

(RR=1.59; 95% CI 0.93-2.72) or women (RR=1.05; 95% CI 0.64-1.71).

74. The borderline statistically significant elevated RR estimate for high as compared to low dietary acrylamide intake reported in the 2015 meta-analysis was based on a fixed effects model, with a lower bound confidence interval of 1.00 (or a null association). In contrast, no statistically significant increase in risk was observed from a random effects model using a continuous measure of acrylamide exposure, or from a summary analysis restricted to never smokers in the meta-analysis. Furthermore, a statistically significant positive association has not been corroborated by subsequent large cohort studies in the United States (McCullough et al. 2019; Graff et al. 2018), which combined included over 1,000 kidney cancer cases. In addition, smoking is a strong and consistent risk factor for kidney cancer (Lipworth et al. 2009) and a major contributor to acrylamide exposure, and thus residual confounding by smoking even after statistical adjustment cannot be ruled out. Residual confounding by smoking in some of the included studies in the meta-analysis may explain the modest, borderline significant result observed in Pelucchi et al. (2015).

75. In my opinion, the collective epidemiologic evidence from several large well-conducted studies, taken together, does not support an association between dietary acrylamide intake and increased risk of kidney cancer.

f. **Prostate Cancer**

76. Only one case-cohort analysis (Perloy et al. 2018) and the 2015 meta-analysis (Pelucchi et al. 2015) have examined the association of dietary acrylamide intake and prostate cancer.

77. Based on more than 13,000 prostate cancer cases included in six studies in various populations, Pelucchi et al. (2015) reported a summary RR of 1.00 (95% CI 0.93-1.08) for the highest vs. lowest category of dietary acrylamide intake as well as for a 10µg/day increase in acrylamide intake (RR=1.00; 95% CI 0.99-1.02). Neither result is statistically significant. All six of the studies included in the 2015 meta-analysis were published prior to the 2012 review by Lipworth et al.

78. A subsequent case-cohort analysis conducted in the NLCS cohort reported on the association between dietary acrylamide intake and advanced prostate cancer, and whether genetic variation modifies that association (Perloy et al. 2018). This analysis was based on 948 cases of advanced prostate cancer identified over 20.3 years of follow-up; these cases overlap with the 741

1 advanced prostate cancer cases that contributed to the previous 13.3-year follow up results from the
2 NLCS (Hogervorst et al. 2008).

3 79. Consistent with the earlier report (Hogervorst et al. 2008), in the updated follow-up
4 (Perloy et al. 2018), no statistically significant association was observed between acrylamide intake
5 and advanced prostate cancer, with a RR of 1.03 (95% CI 0.82-1.29) for the highest vs. lowest
6 quintile. Among never smokers, there was an inverse non-statistically significant association with
7 advanced prostate cancer (RR=0.90; 95% CI 0.51-1.60).

8 80. Additional analyses were conducted of interactions between acrylamide intake and
9 selected genetic variants (58 single nucleotide polymorphisms (SNPs) and 2 gene deletions) in genes
10 involved in acrylamide metabolism or related to DNA repair, sex hormone systems or oxidative
11 stress, mechanisms speculated by the authors to “explain the effect of acrylamide on cancer risk.” An
12 accepted statistical approach when conducting multiple tests of large numbers of genetic variants in
13 large-scale genetic studies is to adjust the significance cut-off in order to reduce the chance of false-
14 positive findings. None of the studied variants showed a statistically significant interaction with
15 acrylamide after applying the adjustment for multiple comparisons. These results indicate that genetic
16 variation does not modify an association between dietary acrylamide and advanced prostate cancer
17 risk.

18 81. In my opinion, the available epidemiologic evidence consistently demonstrates no
19 association between dietary acrylamide intake and prostate cancer overall or advanced prostate
20 cancer.

21 g. **Breast Cancer**

22 82. The 2015 meta-analysis (Pelucchi et al. 2015) and two subsequent cohort studies
23 (Kotemori et al 2018a.; Hogervorst et al. 2019) have examined dietary acrylamide intake in relation
24 to breast cancer.

25 83. The summary RR reported for breast cancer in the 2015 meta-analysis (Pelucchi et al.
26 2015), based on seven studies and 16,773 breast cancer cases, was 0.96 (95% CI 0.91-1.02) for the
27 highest vs. lowest category of dietary acrylamide intake and 1.00 (95% CI 0.98-1.01) for a 10µg/day
28 increase in acrylamide intake. All seven studies included in the 2015 meta-analysis were published

before, and included in, the Lipworth et al. (2012) review.

84. In the subsequent JPHC study (Kotemori et al. 2018a), 792 cases of breast cancer were observed among 48,910 women, with mean follow-up of 15.4 years. The mean level of dietary acrylamide intake was relatively low in this Japanese population, 7.0 μ g/day overall and 11.1 μ g/day in the highest tertile of intake. The RR for the highest vs. lowest tertile of acrylamide intake was 0.95 (95% CI 0.79-1.14), and no statistically significant association was observed for either estrogen receptor positive (ER+) (RR=1.00; 95% CI 0.71-1.40) or ER- breast cancer (RR=0.83; 95% CI 0.51-1.38) or among never smokers (RR=0.93; 95% CI 0.77-1.12).

85. A gene-acrylamide interaction analysis was conducted in the NLCS to examine interactions between acrylamide intake and selected genetic variants (58 single nucleotide polymorphisms (SNPs) and 2 gene deletions) on risk of ER+ breast cancer (Hogervorst et al. 2019). As in the NLCS prostate cancer analysis, the 364 ER+ breast cancer cases in this updated 20.3-year follow-up overlap with those in the earlier report on breast cancer from this cohort (Pedersen et al. 2010).

86. A statistically non-significant inverse association was observed between dietary acrylamide intake and ER+ breast cancer overall, with RRs of 0.85 (95% CI 0.66-1.09) for the highest vs. lowest quintile and 0.94 (95% CI 0.88-1.00) for a 10 μ g/day increase in acrylamide intake. Four of the studied SNPs showed a statistically significant interaction with acrylamide after statistical adjustment for multiple comparisons, but the direction of effect was towards a decreased risk of breast cancer in those with the variant allele and no association with breast cancer for those with wild type allele for all SNPs.

87. In my opinion, neither the meta-analysis nor the two subsequent cohort studies demonstrate any association between acrylamide intake and breast cancer, either overall or by hormone receptor subtype.

h. Ovarian Cancer

88. Since 2012, the Pelucchi et al. (2015) meta-analysis and three additional cohort studies have examined ovarian cancer risk in relation to dietary acrylamide intake (Kotemori et al. 2018b; Hogervorst et al. 2017; Obon-Santacana et al. 2015).

89. The summary RR reported in the 2015 meta-analysis (Pelucchi et al. 2015), based on 2,010 cases observed in one case-control and three cohort studies, was 1.12 (95% CI 0.85-1.47) for the highest vs. lowest category of dietary acrylamide intake and 1.01 (95% CI 0.97-1.05) for a 10µg/day increase in acrylamide intake, and neither result was statistically significant. A non-statistically significant RR of 1.39 (95% CI 0.97-2.00) was observed for ovarian cancer among women who had never smoked. All four studies were published before, and included in, the Lipworth et al. (2012) review.

90. The analysis of ovarian cancer from the EPIC cohort (Obon-Santacana et al. 2015) included a large number of epithelial ovarian cancer cases (N=1,191) observed in a subcohort of 325,006 women followed for an average of 11 years. The relative risk was non-statistically significant and close to the null value for the highest quintile of acrylamide intake vs. the lowest (RR=0.97; 95% CI 0.76-1.23) and for a 10µg/day increase in intake (RR=1.02; 95% CI 0.96-1.09). No statistically significant associations were observed in analyses stratified by ovarian cancer subtype. The authors state that analyses stratified by smoking status did not show an association between ovarian cancer risk and acrylamide intake among never smokers, but data are not presented.

91. As was done for breast cancer, a gene-acrylamide interaction analysis was conducted in the NLCS (Hogervorst et al. 2017) to examine interactions between acrylamide intake and selected genetic variants (58 single nucleotide polymorphisms (SNPs) and 2 gene deletions) on risk of ovarian cancer. Again, the ovarian cancer cases in this updated 20.3-year follow-up overlap with those in the earlier report on ovarian cancer from this cohort (Hogervorst et al. 2007).

92. With the extended follow-up, no statistically significant association was observed between dietary acrylamide intake and ovarian cancer overall, with RRs of 1.38 (95% CI 0.95-1.99) for the highest vs. lowest quintile and 1.06 (95% CI 0.98-1.16) for a 10µg/day increase in acrylamide intake. Among never smokers, the corresponding RR estimates were elevated and statistically significant (RR=1.85; 95% CI 1.15-2.95 for the highest vs. lowest quintile and RR=1.15; 95% CI 1.02-1.30 for a 10µg/day increase in acrylamide intake). This result could have been due to chance, as addressed in paragraph [93] below. There were no statistically significant interactions between acrylamide intake and gene variants after adjustment for multiple genetic comparisons.

93. Finally, in the JPHC (Kotemori et al. 2018b), 122 cases of ovarian cancer were observed after mean follow-up of 15.6 years. Overall, the RR for the highest vs. lowest tertile of acrylamide intake was 0.77 (95% CI 0.49-1.23), and no statistically significant association was observed for the highest tertile of acrylamide intake among never smokers (RR=0.82; 95% CI 0.50-1.33).

94. Two additional nested case-control studies examined the association between acrylamide adduct levels and ovarian cancer risk (Xie et al. 2013; Obon-Santacana et al. 2016b). Neither study reported a statistically significant association between acrylamide adducts and ovarian cancer overall. Among non-smokers, the RRs for the highest vs. lowest category were 0.85 (95% CI 0.57-1.27) in the NHS and 1.19 (95% CI 0.67-2.11) in the EPIC cohort. Neither result was statistically significant.

95. In my opinion, the collective epidemiologic evidence consistently demonstrates no overall association between acrylamide intake and ovarian cancer. Virtually every epidemiologic study of ovarian cancer in relation to acrylamide intake, including the three studies published since the 2015 meta-analysis, has examined the association specifically among non-smoking women, and a statistically significant increase in risk among the subgroup of never smoking women has been reported in only one cohort, the NLCS (Hogervorst et al. 2007, 2017). This result could be a chance finding against a background of many analyses of acrylamide in relation to virtually every cancer type within the NLCS, and it should be interpreted with caution in the absence of confirmation in other large cohort populations.

i. **Endometrial Cancer**

96. Three studies (Obon-Santacana et al. 2014; Pelucchi et al. 2016; Hogervorst et al. 2016), as well as a meta-analysis (Pelucchi et al. 2015) and a pooled analysis (Je 2015), have reported results on dietary acrylamide intake in relation to endometrial cancer since publication of the Lipworth et al. (2012) review. Of the four cohorts included in the pooled endometrial cancer analysis (Je 2015), all are included in the Pelucchi et al. 2015 meta-analysis and three were included in my 2012 review (Hogervorst et al. 2007; Larsson et al. 2009; Wilson et al. 2010).

97. The Pelucchi et al. (2015) meta-analysis and the pooled analysis by Je (2015) both

1 reported summary RRs for four prospective cohort studies. The specific methodologic and analytic
2 approach of the two pooled analyses varied slightly. Pelucchi et al. (2015) reported a non-
3 statistically-significant summary RR for endometrial cancer, based on 2,774 cases, of 1.06 (95% CI
4 0.92-1.23) for the highest vs. lowest category of dietary acrylamide intake and 1.01 (95% CI 0.97-
5 1.06) for a 10µg/day increase in acrylamide intake, with a borderline statistically significant
6 association among never smokers (RR for highest vs. lowest category = 1.23; 95% CI 1.00-1.51).
7 Similarly, based on 2,109 cases, Je (2015) reported a non-statistically-significant pooled RR of 1.10
8 (95% CI 0.91-1.34) for the highest vs. lowest intake and 1.04 (95% CI 0.97-1.11) for a 10µg/day
9 increase in acrylamide intake; corresponding RRs among never-smoking women were 1.39 (95% CI
10 1.09-1.77) and 1.11 (95% CI 1.04-1.19).

11 98. In the European EPIC cohort, which was included in both the meta-analysis (Pelucchi
12 et al. 2015) and the pooled analysis (Je 2015), 1,382 cases of endometrial cancer were observed
13 among 301,113 women followed for over 3 million person-years (Obon-Santacana et al. 2014). No
14 association was observed between endometrial cancer overall and dietary acrylamide intake modeled
15 as highest vs. lowest quintile (overall RR=0.98; 95% CI 0.78-1.25) or per 10µg/day increase (overall
16 RR=0.98; 95% CI 0.92-1.05). Also, no statistically significant association was observed among never
17 smokers (RR for highest vs. lowest quintile=1.01; 95% CI 0.75-1.38) overall. The same was true for
18 analyses restricted to Type I endometrial cancers (endometrioid adenocarcinomas; N=627). In a
19 further small subgroup analysis of Type I endometrial cancer among women who never smoked and
20 did not use oral contraceptives, based on 203 type I cases, the RR for the highest vs. lowest quintile
21 of acrylamide intake was 1.97 (95% CI 1.08-3.62), with a statistically significant dose-response
22 trend.

23 99. For the main analyses of endometrial cancer overall (not limited to Type I), the
24 subgroup of women who never smoked and did not use oral contraceptives did not have a statistically
25 significantly elevated RR associated with high acrylamide intake (RR=1.28; 95% CI 0.88-1.85).
26 Given the large number of comparisons performed in this study for different endometrial cancer
27 subtypes across various strata of smoking, oral contraceptive use and other variables, the single
28 statistically significant subgroup finding for Type I cases could be due to chance and must be

1 evaluated in the context of the collective evidence from all studies.

2 100. Two additional studies have been published since 2015. An Italian case-control study
3 (Pelucchi et al. 2016) of 454 women with endometrial cancer and 908 controls reported a non-
4 statistically significant RR of 1.17 (0.73-1.85) for the highest vs. lowest quintile of acrylamide intake
5 and 1.00 (95% CI 0.91-1.10) for a 10µg/day increase. Among never smokers, the RR for high vs. low
6 acrylamide intake was 1.28 and not statistically significant (95% CI 0.73-2.25).

7 101. An updated case-cohort analysis within the NLCS (Hogervorst et al. 2016) examined
8 interactions between acrylamide intake and selected genetic variants (57 single nucleotide
9 polymorphisms (SNPs) and 2 gene deletions) on risk of endometrial cancer. Again, the 393
10 endometrial cancer cases in this updated 20.3-year follow-up overlap with those in the earlier report
11 on endometrial cancer from this cohort (Hogervorst et al. 2007). In the updated follow-up, there was
12 no statistically significant association between acrylamide intake and endometrial cancer risk, with
13 RRs of 1.03 (95% CI 0.71-1.51) for the highest vs. the lowest quintile and 0.98 (95% CI 0.88-1.10)
14 per 10µg/day increase of intake. Similarly, with longer follow-up, a statistically significant
15 association was no longer observed among never smokers, with RRs of 1.03 (95% CI 0.90-1.18) for
16 the highest vs. the lowest quintile and 1.44 (95% CI 0.90-2.28) per 10µg/day increase of intake.

17 102. For reasons not specified, the authors of Hogervorst et al. 2016 ignored the extended
18 follow-up and restricted the gene-acrylamide interaction analyses to the earlier 11.3-year follow up
19 period. There were no statistically significant interactions between acrylamide intake and gene
20 variants after adjusting for multiple comparisons.

21 103. One additional case-control study (Obon-Santacana et al. 2016a), nested within the
22 EPIC cohort, examined acrylamide and glycidamide adduct levels in relation to endometrial cancer
23 risk among non-smoking postmenopausal women. Neither acrylamide (RR for highest vs. lowest
24 quintile = 0.84; 95% CI 0.49-1.48) nor glycidamide adducts (RR = 0.94; 95% CI 0.54-1.63) were
25 positively associated with risk of endometrial cancer.

26 104. In my opinion, the collective epidemiologic evidence from various populations,
27 including two studies published since the 2015 meta-analysis (Pelucchi et al. 2015), consistently
28 demonstrates no overall association between dietary acrylamide intake and endometrial cancer.

Modest increases in risk of borderline significance among the subgroup of never smoking women have been reported in only two studies (Hogervorst et al. 2007; Obon Santacana et al. 2014) and not in others (Kotemori et al. 2018; Larsson et al. 2009; Wilson et al. 2010; Pelucchi et al. 2016; Hogervorst et al. 2017). Moreover, in the NLCS, the statistically significant association reported in the early report (Hogervorst et al. 2007) was no longer apparent after an additional 10 years of follow-up of the cohort (Hogervorst et al. 2016); and in the EPIC cohort (Obon Santacana et al. 2014), the statistically significant association was observed only for Type I endometrial cancer among women who never smoked and did not use oral contraceptives, one of numerous small subgroup comparisons that could be a chance finding. Thus, this finding requires cautious interpretation in the absence of confirmation in other studies.

j. **Melanoma**

105. A case-cohort analysis (Lipunova et al. 2017) conducted in the NLCS cohort is the only study to date to examine acrylamide intake in relation to cutaneous malignant melanoma. This analysis was based on 501 cases identified over 17.3 years of follow-up. The results indicated no statistically significant association for the highest vs. lowest category of dietary acrylamide intake among men (RR=1.52; 95% CI: 0.98-2.33) or women (RR=0.91; 95% CI: 0.57-1.44). There was a borderline statistically significant increased risk for melanoma for a 10 µg/day increase in acrylamide intake among men (RR= 1.13; 95% CI: 1.01-1.26) but not women (RR= 0.97; 95% CI: 0.86-1.08). However, there was no linear dose response over quintiles of acrylamide intake for risk of melanoma among men ($P_{\text{trend}}=0.12$). The RR for a 10 µg/day increase in acrylamide intake was not significantly elevated among men who had never smoked or had quit for more than 10 years (RR= 1.07; 95% CI: 0.92-1.26) or among women who had never smoked (RR=1.02; 95% CI 0.88-1.20).

106. This single cohort study (NLCS) has evaluated virtually every cancer type in relation to acrylamide intake. The borderline significant slightly elevated subgroup RR estimate reported only for the continuous analysis and only among men, with a lower bound confidence limit of 1.01 and no statistically significant dose-response trend, requires cautious interpretation in the absence of confirmation in other studies. Moreover, direct data were not available on UV exposure, a major cause of malignant melanoma, and residual confounding cannot be ruled out by adjustment for proxy

measures of UV exposure. In my opinion, the epidemiologic evidence does not demonstrate an overall association between dietary acrylamide intake and melanoma.

k. **Lymphatic Malignancies**

107. One case-cohort study (Bongers et al. 2012), which was included in the 2015 meta-analysis (Pelucchi et al. 2015), has examined dietary acrylamide in relation to risk of lymphatic malignancies.

108. In the Pelucchi et al. (2015) meta-analysis, 1,208 cases of lymphoid malignancies were observed in two studies. The summary RR was non-statistically significant and close to the null value for both the highest vs. lowest category of dietary acrylamide intake (RR=1.13; 95% CI 0.89-1.43) and for a 10 µg/day increase in acrylamide intake (RR=1.03; 95% CI 0.99-1.09).

109. The case-cohort analysis (Bongers et al. 2012) conducted in the NLCS cohort reported on the association between dietary acrylamide intake and seven histologic subtypes of lymphatic malignancies in men and women. This analysis was based on a total of 1,233 cases identified over 16.3 years of follow-up, but many of the subtype analyses had small numbers of cases. No statistically significant associations with acrylamide intake were observed in women for any histologic subtype. In men, there was no statistically significant association observed between acrylamide intake and diffuse large cell lymphoma, chronic lymphocytic leukemia, Waldenstrom macroglobulinemia and immunocytoma, mantle cell lymphoma, or T-cell lymphomas. Borderline statistically significant associations were observed in men for a 10µg/day increase in acrylamide intake and both multiple myeloma (RR=1.14; 95% CI: 1.01-1.27 based on 170 cases) and follicular lymphoma (RR=1.28; 95% CI: 1.03-1.61 based on 42 cases); sufficiently large numbers were available to conduct quintile analyses in men for multiple myeloma, yielding a non-significantly increased RR of 1.54 (95% CI 0.92-2.58) for the highest vs. lowest quintile of acrylamide intake.

110. A large number of subgroup analyses, with a limited number of cases, were conducted in this study (Bongers et al. 2012) across not only subtypes of lymphatic malignancies and sex but also combinations of lifestyle variables including smoking; these multiple comparisons are likely to have resulted in a few spurious associations by chance alone. Therefore, the results must be interpreted with caution in the absence of confirmation in other studies. In my opinion, the collective

1 epidemiologic evidence does not demonstrate an association between dietary acrylamide intake and
2 lymphatic malignancies.

3 **V. CONCLUSIONS**

4 111. The association between dietary acrylamide intake and various types of cancer has
5 been extensively studied using different study designs in numerous study populations worldwide,
6 including Europe, the United States, and Asia, with a wide range of dietary acrylamide exposure.

7 112. My review of the collective epidemiologic literature does not reveal any clear or
8 consistent evidence of increased risk of cancer at any site associated with dietary acrylamide intake.
9 In fact, most cancer-specific relative risks have been close to or below the null value (RR=1.0),
10 indicating the absence of an association.

11 113. An occasional statistically significant finding from a single study requires cautious
12 interpretation, given that it likely reflects multiple hypothesis testing in which dozens of cancer
13 endpoints are studied and extensive subgroup analyses are performed in one study. An occasional
14 false positive result by chance alone is to be expected in this scenario because the more analyses that
15 are performed, the higher the likelihood of a statistically significant correlation that is due to chance.

16 114. It is my opinion to a reasonable degree of scientific and epidemiologic certainty that
17 there is no consistent or reliable evidence to support a finding that dietary exposure to acrylamide
18 increases the risk of any type of cancer in humans, either overall or among non-smokers. My opinion
19 is based on my review of the 24 studies published since 2012 as well as the studies published earlier
20 and included in my 2012 review (Lipworth et al. 2012).

21
22 I declare under penalty of perjury under the laws of the United States of America that the
23 foregoing is true and correct, and that this declaration is executed this 8th day of November 2019, in
24 Nashville, TN.


25
26 
27 Loren Lipworth, Sc.D.
28

EXHIBIT A

November 2019

LOREN LIPWORTH, Sc.D.

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Date, Place of Birth: February 13, 1970; Johannesburg, South Africa

Citizenship: United States

EDUCATION

Brown University (Providence, RI), Sc.B., 1991 (Neuroscience, Honors)

Harvard School of Public Health (Boston, MA), Sc.D., 1996 (Epidemiology)
(Thesis: Endogenous hormones in the etiology of breast cancer)

ACADEMIC APPOINTMENTS AND EMPLOYMENT

1989	Research Assistant, Department of Neurosurgery, Columbia University School of Medicine, New York
1989-1991	Laboratory Research Assistant, Department of Neurobiology, Brown University, Providence, Rhode Island
1991-1992	Laboratory Research Assistant, Department of Neurobiology, Rockefeller University, New York, NY
1995-1996	Visiting Lecturer, Department of Cancer Epidemiology, Uppsala University, Uppsala, Sweden
1997-1998	Assistant Professor, Department of Community and Preventive Medicine and Department of Geriatrics and Adult Development, Mount Sinai School of Medicine, New York, NY
1996-1999	Adjunct Assistant Professor, Department of Medical Epidemiology, Karolinska Institute, Stockholm, Sweden
1996-2011	Senior Epidemiologist, International Epidemiology Institute, Rockville, MD
1998-2011	Assistant Professor, Department of Preventive Medicine, Vanderbilt University School of Medicine, Nashville, TN
2011-2018	Research Associate Professor, Division of Epidemiology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN
2018-	Research Professor, Division of Epidemiology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN

EMPLOYMENT

1996-1997 Epidemiologist, Safety Evaluation and Epidemiology, Pfizer Inc, New York, NY

PROFESSIONAL ORGANIZATIONS

Intramural

Vanderbilt Epidemiology Center (VEC)
Vanderbilt-Ingram Cancer Center (VICC), Cancer Epidemiology Research Program
Vanderbilt O'Brien Center for Kidney Disease (VCKD)
Vanderbilt Translational and Clinical Cardiovascular Research Center (V-TRACC)

Extramural

American College of Epidemiology (ACE)
Society for Epidemiologic Research (SER)
American Association for Cancer Research (AACR)
American Heart Association (AHA)
American College of Rheumatology (ACR)

PROFESSIONAL ACTIVITIES

Intramural

2011- Division of Epidemiology Faculty Search Committee
2011- Epidemiology PhD Program Admissions Committee
2011- Epidemiology PhD Program Teaching Faculty
2012- Director, Epidemiology Grand Rounds
2013- Southern Community Cohort Study Data and Biospecimen Use Committee
2014-2017 Southern Community Cohort Study Publications Committee
2014-2019 BioVU Proposal Review Committee
2017- Division of Epidemiology Research Compliance Expert

Extramural

Study Sections

2013-2017 American Heart Association **Genomics and Translational Biology**
Epidemiology and Observational Epidemiology (GTOE) study section

Study Groups

2013- Chair, Molecular Epidemiology Working Group, International Consortium For the Investigation of Renal Malignancies (I-ConFIRM)
2013- Member, Premenopausal Breast Cancer Collaborative Group, NCI Cohort Consortium
2013- Member, Ovarian Cancer Association Consortium (OCAC)
2017- Member, Epidemiology of Endometrial Cancer Consortium (E2C2)
2018- Member, Ovarian Cancer in Women of African Ancestry (OCWAA) Consortium

Editorial responsibilities

Associate Editor: American Journal of Epidemiology, 2010-2014
Ad Hoc Reviewer for numerous journals, including: New England Journal of Medicine, JAMA, Journal of the National Cancer Institute, American Journal of Epidemiology, International Journal of Cancer, Cancer Causes and Control, Cancer, Annals of Oncology, Nutrition and Cancer, European Journal of Epidemiology, Lancet Oncology, Cancer Epidemiology Biomarkers and Prevention, Clinical Epidemiology, Cancer Medicine, Kidney International, Journal of Urology

Honors and Awards

- 1991 Member, Sigma Xi National Honor Society, Brown University
- 1991 Sarah Colver Rosenberger Prize for Outstanding Performance in Neuroscience, Brown University
- 1993-1996 T32CA009001 (PI: Mueller), Training Award in Cancer Epidemiology, National Institutes of Health/National Cancer Institute
- 1994 Honorable Mention, Howard Hughes Predoctoral Fellowship
- 2013 Vanderbilt Epidemiology Center Top 10 Publication of 2013
Sampson UKA, Edwards TL, Jahangir E, Munro H, Wariboko M, Wassef MG, Fazio S, Mensah GA, Kabagambe EK, Blot WJ, Lipworth L. Factors associated with the prevalence of hypertension in the southeastern United States: Insights from 69, 211 blacks and whites in the Southern Community Cohort Study. Circ Cardiovasc Qual Outcomes 2014;7:33-54
- 2017 Vanderbilt Epidemiology Center Top 10 Publication of 2017
Akwo EA, Kabagambe EK, Harrell FE, Blot WJ, Bachmann JM, Wang TJ, Gupta DK*, Lipworth L*. Neighborhood deprivation predicts heart failure risk in a low-income population of blacks and whites in the southeastern United States. Circ Cardiovasc Qual Outcomes 2018;11:e004052

TEACHING ACTIVITIES

- 1989-1991 Department of Neurobiology, Brown University, Teaching Assistant,
- 1994-1996 Department of Epidemiology, Harvard School of Public Health, Teaching Assistant
- 1995-1996 Department of Cancer Epidemiology, Uppsala University, Uppsala, Sweden, Visiting Lecturer – developed and taught introductory epidemiology methods course to first year epidemiology doctoral students
- 2003-2007 Vanderbilt University Medical Center – developed and taught Preventive Medicine course in Cancer Epidemiology to second-year medical students
- 2013- “Obesity, Energy Balance and Cancer,” Vanderbilt Training Program in Molecular and Genetic Epidemiology of Cancer (MAGEC)
- 2015- EPID8370, “Current Topics in Research”, Epidemiology, VUMC
- 2015- BCHM8336, Guest Lecturer, “Epidemiology for the Biochemical and Molecular Toxicologist”, Biochemical and Molecular Toxicology, VU

RESEARCH SUPERVISION

Faculty Mentoring Committees

Margaret Adgent, MSPH, PhD, Research Assistant Professor, Department of Pediatrics/Division of General Pediatrics

Trainees

Trainee	Dates	Research	Current position
Kimberly R. Glenn, MS, PhD <u>PhD Epidemiology, 2014</u>	2011-14	<u>Dissertation:</u> The role of physical activity and obesity in the occurrence of major cardiovascular events among a low-income population with diabetes	Principal Investigator, Lantana Consulting Group; Previously: Director of Healthcare Statistics, TN Department of Health
Elvis A. Akwo, MD, PhD <u>PhD Epidemiology, 2014</u>	2012-17	<u>Dissertation:</u> Disparities in Heart Failure Risk and Survival in the	Post-doctoral Fellow, Division of Nephrology,

		Southern Community Cohort Study	VUMC
Nathaniel Mercaldo, PhD <u>PhD Biostatistics, 2017</u>	2015-17	<u>Dissertation:</u> Outcome-dependent sampling designs for longitudinal ordinal and binary response data	Instructor, Statistics and Radiology, Mass General Hospital and Harvard Medical School
Tian Shen, MD	2013-15	Vanderbilt-Shanghai Chronic Disease Research Training Program, Risk factors for renal cell carcinoma in the Shanghai Men's and Women's Health Studies	Epidemiology PhD Candidate, Shanghai
Nicholas Conley	2014	Summer Program in Integrative Science and Cancer Research	Medical Student, Meharry Medical College
Galen Shi	2014-15	VICTR Summer Research Program, Identification and Characterization of Heterozygous Familial Hypercholesterolemia Patients Using the Synthetic Derivative database	Medical Student, Johns Hopkins University Medical School
Thomas Overton	2015	Summer Program in Integrative Science and Cancer Research	Medical Student, Meharry Medical College
Jai Singh, MD	2014-17	Non-laboratory-based CVD risk score in predicting CVD mortality in the SCCS; Life's Simple 7 and incident cancer in the SCCS	Instructor in Medicine, Division of Cardiovascular Medicine, VUMC
Jacob Taylor, PhD, RD	2016-18	Gene-diet interactions in ESRD; Diet and physical activity in relation to ESRD in the SCCS	Post-doctoral Fellow, Division of Nephrology, VUMC
Sudipa Sarkar, MD	2016-17	Risk factors for non-alcoholic fatty liver disease in the SCCS	Assistant Professor of Medicine, Johns Hopkins
Fabian Bock, MD	2016-	Analgesics in relation to ESRD in the SCCS; Prevalence and racial disparities of CKD in the SCCS	Resident Physician, Harrison Society Scholar – Nephrology, Dept of Internal Medicine, VUMC
Devika Nair, MD	2016-	Psychosocial risk factors for ESRD in the SCCS	Nephrology Fellow, Clinical Research Track, VUMC
Benson Hamooya, MS	2017-	Virological failure among HIV patients with metabolic syndrome receiving antiretroviral therapy in Zambia	PhD Candidate, NIH/Fogarty UNZA-Vanderbilt Training Partnership for HIV-Nutrition-Metabolic Research (UVP)
Blanaid Hicks, PhD (Queens University Belfast)	2016-	An investigation of potential biomarkers and modifiable factors for renal cell carcinoma risk and survival	Postdoctoral Fellow, Cancer Research UK
Debra Dixon, MD	2017-	Psychosocial risk factors for heart failure in the SCCS	Resident Physician, Harrison Society Scholar – Cardiology, Dept of Internal Medicine, VUMC
Danielle Kubicki	2018-	Modifiable risk factors and heart failure in the SCCS	Second year medical student, VU School of Medicine Research Immersion Program

Mindy Pike, MPH	2018-		Epidemiology PhD student
Meredith Duncan	2018-	Lifetime Smoking and its Impact on Risk for Cardiovascular Disease and Lung Cancer: Results from the Framingham Heart Study	Epidemiology PhD student
Jaleesa Moore, DrPh	2018-	Racial disparities in cancer	Post-doctoral Fellow, Molecular and Genetic Epidemiology of Cancer (T32) Training Program

RESEARCH INTERESTS

Role of obesity and other metabolic traits in the etiology and racial disparities of cancer and cardiovascular disease; metabolomic profiling of diabetes, cardiovascular and renal diseases and cancer; epidemiology of kidney cancer and kidney diseases, including racial disparities and gene-environment interactions; early life risk factors for obesity and other cardiometabolic traits and adult disease; pharmacoepidemiology, pharmacogenomics, and health effects of medications and medical devices; epidemiology; epidemiologic methods

RESEARCH SUPPORT

Active

1R01RDK081572-09 (WANG) **08/01/16-07/31/20** **Co-Investigator**

NIH/NIDDK/Beth Israel: Metabolomic predictors of insulin resistance and diabetes

To examine whether metabolite profiling in well-phenotyped populations will illuminate T2D-associated metabolic pathways among African Americans.

5R01 DK108159-04 (WANG) **04/01/16-03/31/20** **Co-Investigator**

NIH/NIDDK: Metabolite profiles and the risk of diabetes in Asians

The majority of diabetes cases worldwide in the next decade are expected to occur among Asian individuals, and Asians in this country have high rates of developing diabetes as well. Studying why Asians, particularly lean Asians, are particularly susceptible to diabetes should enhance our biological understanding of the disease and facilitate the development of strategies for prevention and treatment.

1P30DK114809-01 (HARRIS) **07/01/17-06/30/22** **Co-Investigator**

NIH/NIDDK: Vanderbilt O'Brien Kidney Center

The mission of the Vanderbilt O'Brien Kidney Center (VOKC) is to advance research opportunities for kidney-related research and promote effective interactions between basic scientists and clinical researchers in order to advance effective prevention and treatment of acute and chronic kidney disease and their complications.

1R01HL133870-02 (WANG) **04/01/17-06/30/21** **Co-Investigator**

NIH/NHLBI/Univ of Miss: Aptamer Proteomics of Cardiometabolic and Renal Traits in African Americans

There is a disproportionate burden of metabolic, cardiovascular, and renal disease among African Americans, but the responsible environmental and/or genetic mechanisms are incompletely defined. We will use powerful new techniques to study blood plasma, seeking proteins that

indicate disease risk or provide hints regarding disease mechanisms. These studies may lead to earlier treatment and to the development of new medicines.

1R01DK117114-01A1 (FERGUSON) 8/01/18-7/31/23 Co-Investigator
NIH/NDDK: Determinants of alpha-amino adipic acid (2-AAA) and relationship to diabetes
Diabetes is one of the leading causes of death in the US and worldwide. We have identified a new diabetes risk marker in blood, alpha-amino adipic acid (2-AAA), which predicts development of diabetes, and might cause disease development. Because very little is known about 2-AAA, we will study the dietary and genetic factors that determine high or low 2-AAA, to understand whether we can use knowledge about 2-AAA to improve prediction and treatment of diabetes.

1R01HL142856-01A1 (FERGUSON) 4/01/19-3/31/24 Co-Investigator
NIH/NHLBI: Virtual metabolomics as a discovery tool for novel cardiometabolic disease biology
This genetics-based “virtual” metabolite study design that will allow us to define genetic predictors of metabolite concentrations in a small population in whom the metabolite was measured, and then use these genetic predictors to impute metabolite concentrations in a large population in whom the metabolite was not measured.

1R01DK122075-01 (ROBINSON-COHEN) 7/16/19-6/30/24 Co-Investigator
NIH/NIDDK: investigating causality between abnormalities of mineral metabolism and kidney, cardiovascular and bone disease
The goal of this application is to comprehensively evaluate causal relationships between mineral metabolites, kidney function, treatment strategies and clinical and subclinical phenotypes of cardiovascular and bone disease. We will conduct analyses using genomic data, based on Mendelian Randomization techniques, and will leverage publicly available genome-wide association data and two of the largest practice-based biobanks in the world: Vanderbilt’s BioVU and the Million Veteran Program (MVP).

5U01CA202979-03 (BLOT) 07/01/2016-06/30/2021 Co-Investigator
NIH/NCI: Southern Community Cohort Study
In this application we aim to develop a suite of statistical and computational methods to identify genes and variants associated with complex disease and use BioVU, a DNA BioBank linked to electronic health records, to validate and investigate genetic architecture of multiple complex diseases.

R03AA026099 (LIPWORTH) 09/01/18-08/31/19 PI
NIH/NIAA/Ochsner: Interaction between alcohol, statins and cardiovascular risk

5 UL1 TR002243-02 (BERNARD) 06/27/12-05/31/19 Co-Investigator
NIH/NCATS: The Vanderbilt Institute for Clinical and Translational Research (VICTR)
VICTR is focused on removing impediments to research translation; creating new infrastructure, training new C&T scientists, and engaging and involving the local community to improve health.

VUMC77746 (LIPWORTH) 10/01/19-09/30/20 PI
EpidStrategies: Heart Failure

To identify real-world cohorts of patients with a diagnosis of heart failure with reduced ejection fraction using electronic health records, and to describe their clinical, laboratory and health outcome characteristics.

Pending

R01 (MPI: LIPWORTH, IKIZLER)

NIH/NIDDK: Interactions of APOL1 with genetic variants and acidosis in end-stage kidney disease in African Americans

R01HL (LIPWORTH)

NIH/NHLBI (Sub: Mass General-PI: Leong): Towards Precision Care of Patients with Extreme Genetic Risk for Type 2 Diabetes

R01CA230337 (LIPWORTH)

NIH/NCI (Sub: Harvard/USC/MSKCC; MPI: De Vivo, Du, Setiawan): Comprehensive molecular characterization of endometrial cancer, etiologic heterogeneity, and racial disparities

R01HL (YU)

NIH/NHLBI: Gut microbial metabolites and risk of coronary heart disease: a prospective, multiethnic, metabolomic study
Role: Co-Investigator

R01CA (SHU)

NIH/NCI: Triple negative breast cancer prognosis: Evaluation of gene expression and immune predictors
Role: Co-Investigator

Completed

5R03 CA192214-02 (LIPWORTH)

07/01/15-06/30/17

PI

NIH/NCI: Understanding Breast Cancer Subtypes in Black Women

The goal of this study is to collect and classify breast tumor tissue among black women to allow for detailed characterization of intrinsic molecular subtypes.

5P50 CA098131-15 (PIETENPOL)

09/17/13-08/31/18

Co-Investigator/Project Leader

NIH/NCI: SPORE in Breast Cancer

The obesity-metabolic biomarker axis and breast cancer risk. Projects will transform how we diagnose and treat individuals with breast cancer and deepen our understanding of the pathobiology of mammary neoplasia.

VUMC57013, (LIPWORTH)

06/01/16-05/31/18

PI

AstraZeneca AB

SUPREME-HN: A Retrospective Cohort Study of PD-L1 in Recurrent and Metastatic Squamous Cell Carcinoma of Head and Neck

VUMC Internal Project, (LIPWORTH)

07/01/15-06/30/16

PI

Vanderbilt Center for Kidney Diseases: Examining environmental exposures to explain kidney disease racial disparities

EpidStat Institute, (LIPWORTH) 06/01/14-05/31/16 PI
Identification and Characterization of Heterozygous Familial Hypercholesterolemia Patients Using the Vanderbilt University Medical Center Synthetic Derivative database

5P30ES000267-47, (PI: RIZZO) 05/02/10-03/31/16 Co-Investigator
NIH/NIEHS: Center in Molecular Toxicology
The goal of this pilot study is to examine whether exposures to trace metals explains kidney disease racial disparities.

EpidStat Institute, (LIPWORTH) 06/01/13-08/31/14 PI
Identifying and characterizing hyperlipidemic statin-treated patients secondary to diagnosis of diabetes or a cardiovascular event

R03CA081590, (LIPWORTH) 9/1/1999-8/31/2002 PI
NIH/NCI
Infectious etiology of testicular cancer
This study evaluated the association between antibody response to HSV-2, HPV-16 and 15, HHV-8 and Chlamydia trachomatis and the occurrence of testicular cancer.

T32CA009001, (MUELLER) 9/1/1993-8/31/1996
NIH/NCI
Program for training in cancer epidemiology
Role: Predoctoral training grant recipient

PUBLICATIONS

Articles in refereed journals

1. Lipworth L. Epidemiology of breast cancer. Eur J Cancer Prev 1995;4:7-30.
2. Lipworth L, Katsouyanni K, Ekbohm A, Michels KB, Trichopoulos D. Abortion and the risk of breast cancer: a case-control study in Greece. Int J Cancer 1995;61:181-184.
3. Lipworth L, Katsouyanni K, Stuver S, Samoli E, Hankinson S, Trichopoulos D. Oral contraceptives, menopausal estrogens, and the risk of breast cancer: a case-control study in Greece. Int J Cancer 1995;62:548-551.
4. Bernstein L, Lipworth L, Ross RK, Trichopoulos D. Correlation of estrogen levels between successive pregnancies. Am J Epidemiol 1995;142:625-628.
5. La Vecchia C, Negri E, Franceschi S, Decarli A, Giacosa A, Lipworth L. Olive oil, other dietary fats and the risk of breast cancer. Cancer Causes Control 1995;6:545-550.
6. Adami H-O, Lipworth L, Titus-Ernstoff L, Hsieh C-C, Hanberg A, Ahlborg U, Baron J, Trichopoulos D. Organochlorine compounds and estrogen-related cancers in women. Cancer Causes Control 1995;6:551-556.
7. Ahlborg U, Lipworth L, Titus-Ernstoff L, Hsieh C-c, Hanberg A, Baron J, Trichopoulos D, Adami H-O. Organochlorine compounds in relation to breast cancer, endometrial cancer and endometriosis: an assessment of the biologic and epidemiologic evidence. Crit Rev Toxicol 1995;25:463-531.

8. Trichopoulou A, Kouris-Blazos A, Wahlqvist ML, Gnardellis C, Lagiou P, Polychronopoulos E, Vassilakou T, Lipworth L, Trichopoulos D. Diet and overall survival of the elderly. Br Med J 1995;311:1457-1460.
9. Trichopoulos D, Lipworth L. Is cancer causation simpler than we thought, but more intractable? (editorial) Epidemiology 1995;6:347-349.
10. Lipworth L, Adami H-O, Trichopoulos D, Carlström K, Mantzoros C. Serum steroid hormones, sex hormone-binding globulin and body mass index in the etiology of postmenopausal breast cancer. Epidemiology 1996;7:96-100.
11. Katsouyanni K, Lipworth L, Trichopoulou A, Samoli E, Stuver S, Trichopoulos D. A case-control study of lactation and cancer of the breast. Br J Cancer 1996;73:814-818.
12. Thurfjell E, Hsieh C-c, Lipworth L, Ekblom A, Trichopoulos D, Adami H-O. Breast size and mammographic pattern in relation to breast cancer risk. Eur J Cancer Prev 1996;5:37-41.
13. Ekblom A, Hsieh C-c, Lipworth L, Wolk A, Pontén J, Adami H-O, Trichopoulos D. Perinatal characteristics in relation to prostate cancer incidence and mortality. Br Med J 1996;313:337-341.
14. Tzonou A, Lipworth L, Kalandidi A, Trichopoulou A, Gamatsi I, Hsieh C-c, Notara V, Trichopoulos D. Dietary factors and the risk of endometrial cancer: a case-control study in Greece. Br J Cancer 1996;73:1284-1290.
15. Kalandidi A, Tzonou A, Lipworth L, Gamatsi I, Filippa D, Trichopoulos D. A case-control study of endometrial cancer in relation to reproductive, somatometric and lifestyle variables. Oncology 1996;53:354-359.
16. Garidou A, Tzonou A, Lipworth L, Signorello LB, Kalapothaki V, Trichopoulos D. Lifestyle factors and medical conditions in relation to esophageal cancer by histologic type in a low-risk population. Int J Cancer 1996;68:295-299.
17. Tzonou A, Lipworth L, Garidou A, Signorello LB, Lagiou P, Hsieh C-c, Trichopoulos D. Diet and the risk of esophageal cancer by histologic type in a low-risk population. Int J Cancer 1996;68:300-304.
18. Agapitos E, Mollo F, Tomatis L, Katsouyanni K, Lipworth L, Delsedime L, Kalandidi A, Karakatsani A, Riboli E, Saracci R, Trichopoulos D. Epithelial, possibly precancerous, lesions of the lung in relation to smoking, passive smoking, and socio-demographic variables. Scand J Soc Med 1996;4:259-263.
19. Ekblom A, Hsieh C-c, Lipworth L, Adami H-O, Trichopoulos D. Intra-uterine environment and breast cancer risk in women: a population-based study. J Natl Cancer Inst 1997;88:71-76.
20. Lipworth L, Martinez ME, Angell J, Hsieh C-c, Trichopoulos D. Olive oil and human cancer: an assessment of the evidence. Prev Med 1997;26:181-190.
21. Trichopoulou A, Georgiou E, Bassiakos Y, Lipworth L, Proukakis C, Trichopoulos D. Energy intake and monounsaturated fat in relation to bone mineral content among women and men in Greece. Prev Med 1997;26:395-400.
22. Trichopoulos D, Lipworth L. Epidemiology and medicine. Orgyn 1997;3:44-47.

23. Signorello LB, Tzonou A, Mantzoros CS, Lipworth L, Laggiou P, Hsieh C-c, Stampfer M, Trichopoulos D. Serum steroids in relation to prostate cancer risk in a case-control study (Greece). Cancer Causes Control 1997;8:632-636.
24. Seretakis D, Laggiou P, Lipworth L, Signorello L, Trichopoulos D. Changing seasonality of coronary mortality in the USA. JAMA 1997;278:1012-1014.
25. Laggiou P, Mantzoros CS, Tzonou A, Signorello LB, Lipworth L, Trichopoulos D. Serum steroids in relation to benign prostatic hyperplasia. Oncology 1997;54:497-501.
26. Cnattingius S, Bergström R, Lipworth L, Kramer MS. Prepregnancy weight and the risk of adverse pregnancy outcomes. New Engl J Med 1998;338:147-152.
27. Ros HS, Cnattingius S, Lipworth L. A comparison of risk factors for preeclampsia and gestational hypertension in a population-based cohort study. Am J Epidemiol 1998;147:1062-1070.
28. McLaughlin JK, Lipworth L, Chow W-H, Blot WJ. Analgesic use and chronic renal failure: a critical review of the epidemiologic literature. Kidney Int 1998;54:679-686.
29. Hsieh C-c, Signorello LB, Lipworth L, Laggiou P, Mantzoros CS, Trichopoulos D. Predictors of sex hormone levels among the elderly: a study in Greece. J Clin Epidemiol 1998;51:837-841.
30. Lipworth L, Hsieh C-c, Wide L, Ekblom A, Yu S-z, Yu G-p, Xu B, Hellerstein S, Carlström K, Trichopoulos D, Adami H-O. Maternal pregnancy hormone levels in an area with high incidence (Boston, USA) and low incidence (Shanghai, China) of breast cancer. Br J Cancer 1999;79:7-12.
31. Akre O, Lipworth L, Cnattingius S, Sparén P, Ekblom A. Risk factor patterns for cryptorchidism and hypospadias. Epidemiol 1999;10:364-369.
32. Akre O, Lipworth L, Tretli S, Linde A, Engstrand L, Adami H-O, Melbye M, Andersen A, Ekblom A. Epstein-Barr virus in relation to testicular cancer risk: a nested case-control study. Int J Cancer 1999;82:1-5.
33. Olsen JH, McLaughlin JK, Nyren O, Mellemkjaer L, Lipworth L, Blot W. Hip and knee implantations among patients with osteoarthritis and risk of cancer: a record-linkage study from Denmark. Int J Cancer 1999;81:719-722.
34. McLaughlin JK, Lipworth L. A critical review of the epidemiologic literature on health effects of occupational exposure to vinyl chloride. J Epidemiol Biostat 1999;4:253-275.
35. Lipworth L, Bailey LR. History of breastfeeding in relation to breast cancer risk: a review of the epidemiologic literature. J Natl Cancer Inst 2000;92:302-312.
36. McLaughlin JK, Lipworth L. Epidemiologic aspects of renal cell cancer. Semin Oncol 2000;27:115-123.
37. Ros HS, Lichtenstein P, Lipworth L, Cnattingius S. Genetic effects on the liability of developing pre-eclampsia and gestational hypertension. Am J Med Genetics 2000;91:256-260.
38. Fryzek JP, Lipworth L, Garabrant DH, McLaughlin JK. Comparison of surrogate with self-respondents for occupational factors. J Occup Environ Med 2000;42:194-199.
39. Fryzek JP, Weiderpass E, Signorello LB, Hakelius L, Lipworth L, Blot WJ, McLaughlin JK, Nyren O. Characteristics of women with cosmetic breast augmentation surgery compared with breast reduction surgery patients and women in the general population of Sweden. Ann Plast Surg 2000;45:349-356.

40. Lipworth L, Hsieh C-C. Maternal pregnancy hormone profiles in areas with a different incidence of breast cancer. *Br J Cancer* 2000;83:1255-1256.
41. Fryzek JP, Signorello LB, Hakelius L, Lipworth L, McLaughlin JK, Blot WJ, Nyren O. Local complications and subsequent symptom reporting among women with cosmetic breast implants. *Plast Reconstr Surg* 2001;107:214-221.
42. Winther JF, Friis S, Bach FW, Mellekjaer L, Kjoller K, McLaughlin JK, Lipworth L, Blot WJ, Olsen JH. Neurologic disease among women with silicone breast implants in Denmark. *Acta Neurol Scand* 2001;103:93-96.
43. Hansen J, Raaschou-Nielsen O, Christensen JM, Johansen I, McLaughlin JK, Lipworth L, Blot W, Olsen JH. Cancer incidence among Danish workers exposed to trichloroethylene. *J Occup Environ Med* 2001;43:133-139.
44. Kjoller K, Friis S, Mellekjaer L, McLaughlin JK, Winther JF, Lipworth L, Blot WJ, Fryzek JP, Olsen JG. Connective tissue disease and other rheumatic conditions following cosmetic breast implantation in Denmark. *Arch Intern Med* 2001;161:973-979.
45. Lipworth L, Fryzek JP, Forel CM, Blot WJ, McLaughlin JK. Comparison of surrogate with self-respondents regarding medical history and prior medication use. *Int J Epidemiol* 2001;30:303-308.
46. McLaughlin JK, Lipworth L, Marano DE, Tarone R. A critical examination of the scientific basis of the MAK Commission's new general threshold limit values for dust. *Int Arch Occup Environ Health* 2001;74:303-314.
47. Signorello LB, Ye W, Fryzek JP, Lipworth L, Fraumeni JR, JF, Blot WJ, McLaughlin JK, Nyren O. A nationwide study of cancer risk among hip replacement patients in Sweden. *J Natl Cancer Inst* 2001;93:1405-1410.
48. Hernan MA, Zhang S, Lipworth L, Olek MJ, Ascherio A. Multiple sclerosis and age at infection with common viruses. *Epidemiol* 2001;12:301-306.
49. Jensen B, Bliddal H, Kjoller K, Wittrup IH, Friis S, Hoier-Madsen M, Rogind H, McLaughlin JK, Lipworth L, Danneskiold-Samsøe B, Olsen JH. Rheumatic manifestations in Danish women with silicone breast implants. *Clin Rheumatol* 2001;20:345-352.
50. Kjoller K, Holmich L, Jacobsen PH, Friis S, Fryzek JP, McLaughlin JK, Lipworth L, Henriksen TF, Jorgensen A, Bittman S, Olsen JH. Capsular contracture after cosmetic breast implant surgery in Denmark. *Ann Plast Surg* 2001;47:359-366.
51. Holmich LR, Kjoller K, Vejborg I, Conrad C, Sletting S, McLaughlin JK, Fryzek J, Breiting V, Jorgensen A, Olsen JH, Lipworth L, Jacobsen PH, Brandt B. Prevalence of silicone breast implant rupture among Danish women. *Plast Reconstr Surg* 2001;108:848-858.
52. Forel CM, Ejerblad E, Lindblad P, Fryzek JP, Dickman PW, Signorello LB, Lipworth L, Elinder CG, Blot WJ, McLaughlin JK, Zack MM, Nyren O. Acetaminophen, aspirin, and chronic renal failure. *N Engl J Med* 2001;345:1801-1808.
53. Fryzek JP, Lipworth L, Signorello LB, McLaughlin JK. The reliability of dietary data for self and next-of-kin respondents. *Ann Epidemiol* 2002;12:278-283.

54. Friis S, Nielsen GL, Mellemkjaer L, McLaughlin JK, Thulstrup AM, Blot WJ, Lipworth L, Vilstrup H, Olsen JH. Cancer risk in persons receiving prescriptions for paracetamol: a Danish cohort study. Int J Cancer 2002;97:96-101.
55. Kjoller K, Holmich L, Jacobsen PH, Friis S, Fryzek JP, McLaughlin JK, Lipworth L, Henriksen TF, Jorgensen A, Bittman S, Olsen JH. Epidemiologic investigation of local complications after cosmetic breast implant surgery in Denmark. Ann Plast Surg 2002;48:229-237.
56. Kjoller K, Friis S, Signorello LB, McLaughlin JK, Blot WJ, Lipworth L, Mellemkjaer L, Winther JF, Olsen JH. Health outcomes in offspring of Danish mothers with cosmetic breast implants. Ann Plast Surg 2002;48:238-245.
57. Signorello LB, McLaughlin JK, Lipworth L, Friis S, Sorensen HT, Blot WJ. Confounding by indication in epidemiologic studies of commonly used analgesics. Am J Ther 2002;9:199-205.
58. Raaschou-Nielsen O, Hansen J, Thomsen BL, Johansen I, Lipworth L, McLaughlin JK, Olsen JH. Exposure of Danish workers to trichloroethylene, 1947-1989. Appl Occup Environ Hyg 2002;17:693-703.
59. Fryzek JP, Ye W, Signorello LB, Lipworth L, Blot WJ, McLaughlin JK, Nyren O. The incidence of cancer among patients with knee implantations in Sweden, 1980-1994. Cancer 2002;94:3057-3062.
60. Wu J, Hellerstein S, Lipworth L, Wide L, Xu B, Yu G-p, Kuper HE, Laggiou P, Hankinson SE, Ekblom A, Carlstrom K, Trichopoulos D, Adami H-O, Hsieh C-C. Correlates of pregnancy oestrogen, progesterone and sex hormone binding globulin in the USA and China. Eur J Cancer Prev 2002;11:283-293.
61. Lipworth L, Johansen C, Arnsbo P, Moller M, McLaughlin JK, Olsen JH. Cancer risk among pacemaker recipients in Denmark, 1982-1996. J Long-Term Effects Med Implants 2002;12:263-270.
62. Signorello LB, Ye W, Fryzek JP, Blot WJ, Lipworth L, McLaughlin JK, Nyren O. A nationwide followup study of autoimmune and connective tissue disease among hip and knee implant patients. J Long-Term Effects Med Implants 2002;12:255-262.
63. Kjoller K, Holmich LR, Fryzek JP, Jacobsen PH, Friis S, McLaughlin JK, Lipworth L, Henriksen TF, Jorgensen S, Bittmann S, Olsen JH. Characteristics of women with cosmetic breast implants compared with women with other types of cosmetic surgery and population-based controls in Denmark. Ann Plast Surg 2003;50:6-12.
64. Jepsen P, Skriver MV, Floyd A, Lipworth L, Schonheyder HC, Sorensen HT. A population-based study of maternal use of amoxicillin and pregnancy outcome in Denmark. Br J Clin Pharmacol 2003;55:216-221.
65. Xu B, Lipworth L, Wide L, Wu J, Yu S-z, Laggiou P, Kuper HE, Hankinson SE, Carlstrom K, Adami H-O, Trichopoulos D, Hsieh C-C. Maternal and gestational correlates of pregnancy prolactin and growth hormone in USA and China. Eur J Cancer Prev 2003;12:35-42.
66. Signorello LB, McLaughlin JK, Lipworth L, Friis S, Sørensen HT, Blot WJ. Confounding by indication: implications for implant and drug research. J Long-Term Effects Med Implants 2003;13:63-68.

67. Lipworth L, Friis S, Mellekjaer L, Signorello LB, Johnsen SP, Nielsen GL, Sorensen HT, Olsen JH, McLaughlin JK, Blot WJ. A population-based cohort study of mortality among adults prescribed paracetamol in Denmark. J Clin Epidemiol 2003;56:796-801.
68. Pukkala E, Kulmala I, Hovi SL, Hemminki E, Keskimäki I, Lipworth L, Boice JD, McLaughlin JK. Causes of death among Finnish women with cosmetic breast implants, 1971-2001. Ann Plast Surg 2003;51:339-342.
69. Bosetti C, La Vecchia C, Lipworth L, McLaughlin JK. Occupational exposure to vinyl chloride and cancer risk: a review of the epidemiologic literature. Eur J Cancer Prev 2003;12:427-430.
70. Pukkala E, Kulmala I, Hovi SL, Hemminki E, Keskimäki I, Lipworth L, Boice JD, McLaughlin JK. Causes of death among Finnish women with cosmetic breast implants, 1971-2001. Ann Plast Surg 2003;51:339-342.
71. McLaughlin JK, Lipworth L, Tarone RE. Suicide among women with cosmetic breast implants: a review of the epidemiologic evidence. J Long Term Eff Med Implants 2003;13:445-450.
72. Kjoller K, Holmich LR, Fryzek JP, Jacobsen PH, Friis S, McLaughlin JK, Lipworth L, Henriksen TF, Hoier-Madsen M, Wiik A, Olsen JH. Self-reported musculoskeletal symptoms among Danish women with cosmetic breast implants. Ann Plast Surg 2004;52:1-7.
73. McLaughlin JK, Lipworth L. Brain cancer and cosmetic breast implants: a review of the epidemiologic evidence. Ann Plast Surg 2004;52:115-117.
74. Lipworth L, Tarone RE, McLaughlin JK. Breast implants and fibromyalgia: a review of the epidemiologic evidence. Ann Plast Surg 2004;54:284-287.
75. Lipworth L, Friis S, Blot WJ, McLaughlin JK, Mellekjaer L, Johnsen SP, Norgaard B, Olsen JH. A population-based cohort study of mortality among users of ibuprofen in Denmark. Am J Ther 2004;11:156-163.
76. Lipworth L, Tarone RE, McLaughlin JK. Silicone breast implants and connective tissue disease: an updated review of the epidemiologic evidence. Ann Plast Surg 2004;52:598-601.
77. McLaughlin JK, Wise TN, **Lipworth L**. Increased risk of suicide among patients with breast implants: do the epidemiologic data support psychiatric consultation? Psychosomatics 2004;45:277-80.
78. Tarone RE, Lipworth L, Young VL, McLaughlin JK. Breast reduction surgery and breast cancer risk: does reduction mammoplasty have a role in primary prevention strategies for women at high risk of breast cancer? Plast Reconstr Surg 2004;113:2104-2110.
79. Kulmala I, McLaughlin JK, Pakkanen M, Lassila K, Holmich LR, Lipworth L, Boice JD Jr, Raitanen J, Luoto R. Local complications after cosmetic breast implant surgery in Finland. Ann Plast Surg 2004;53:413-419.
80. Blot WJ, Fischer T, Nielsen GL, Friis S, Mumma M, Lipworth L, DuBois R, McLaughlin JK, Sorensen HT. Outcome of upper gastro-intestinal bleeding and use of ibuprofen versus paracetamol. Pharm World Sci 2004;26:319-323.
81. Fryzek JP, Hansen J, Cohen S, Bonde JP, Llambras MT, Kolstad HA, Skytthe A, Lipworth L, Blot WJ, Olsen JH. A cohort study of Parkinson's disease and other neurodegenerative disorders in Danish welders. J Occup Environ Med 2005;47:466-472.

82. McLaughlin JK, Signorello LB, Lipworth L. Confounding by indication, protopathic bias, data dredging and false positive associations in epidemiologic studies of analgesics and disease. Fam Pract Recertification 2005;27:23-29.
83. Fryzek JP, Ye W, Nyrén O, Tarone RE, Lipworth L, McLaughlin JK. A nationwide epidemiologic study of breast cancer incidence following breast reduction surgery in a large cohort of Swedish women. Breast Cancer Res Treat 2006;97:131-134.
84. Fryzek JP, Poulsen AH, Lipworth L, Pedersen L, Nørgaard M, McLaughlin JK, Friis S. A cohort study of antihypertensive medication use and breast cancer among Danish women. Breast Cancer Res Treat 2006;97:231-236.
85. Lipworth L, Tarone RE, McLaughlin JK. The epidemiology of renal cell carcinoma. J Urol 2006;176:2353-2358.
86. McLaughlin JK, Lipworth L, Fryzek JP, Ye W, Tarone RE, Nyrén O. Long-term cancer risk among Swedish women with silicone breast implants: an update of a nationwide study. J Natl Cancer Inst 2006;98:557-560.
87. McLaughlin JK, Lipworth L, Tarone RE. Epidemiologic aspects of renal cell carcinoma. Sem Oncol 2006;33:527-533.
88. Wogelius P, Norgaard M, Gislum M, Pedersen L, Munk E, Mortensen PB, Lipworth L, Sorensen HT. Maternal use of selective serotonin reuptake inhibitors and risk of congenital malformations. Epidemiol 2006;17:701-704.
89. Lipworth L, Nyren O, Ye W, Fryzek JP, Tarone RE, McLaughlin JK. Excess mortality from suicide and other external causes of death among women with cosmetic breast implants. Ann Plast Surg 2007; 59:119-123; discussion 124-5.
90. Fryzek JP, Hölmich L, McLaughlin JK, Lipworth L, Tarone RE, Henriksen T, Kjøller K, Friis S. A nationwide study of connective tissue disease and other rheumatic conditions among Danish women with long-term cosmetic breast implantation. Ann Epidemiol 2007; 17:374-9.
91. McLaughlin JK, Lipworth L, Murphy DK, Walker PS. The safety of silicone gel-filled breast implants: a review of the epidemiologic evidence. Ann Plast Surg 59:569-580, 2007.
92. Kjøller K, Friis S, Lipworth L, McLaughlin JK, Olsen J. Adverse health outcomes in offspring of mothers with cosmetic breast implants: a review. Plast Reconstr Surg 2007;120 (Suppl. 1):129S-134S.
93. McLaughlin JK, Lipworth L, Tarone RE. Epidemiology of renal cancer. Kidney Cancer J 2007;5:10-14.
94. Hölmich L, Lipworth L, McLaughlin JK, Friis S. Breast implant rupture and connective tissue disease - a review of the literature. Plast Reconstr Surg 2007;120 (Suppl. 1):62S-69S.
95. Boffetta P, McLaughlin JK, La Vecchia C, Tarone RE, Lipworth L, Blot WJ. False-positive results in cancer epidemiology: a plea for epistemological modesty. J Natl Cancer Inst 2008;100:988-995.
96. Lamberg S, Manninen M, Kulmala I, McLaughlin JK, Lipworth L, Pakkanen M, Luoto R. Health-related quality of life issues after cosmetic breast implant surgery in Finland. Ann Plastic Surg 2008;61:485-488.
97. Fei C, McLaughlin JK, Lipworth L, Olsen J. Prenatal exposure to PFOA and PFOS and maternally reported developmental milestones in infancy. Environ Health Perspect 2008;116:1391-1395.

98. Halldorsson TI, Fei C, Olsen J, Lipworth L, McLaughlin JK, Olsen SF. Dietary predictors of perfluorinated chemicals: a study from the Danish National Birth Cohort. Environ Sci Technol 2008;42:8971-8977.
99. Lipworth L, Tarone RE, Friis S, Ye W, Olsen J, Nyren O, McLaughlin JK. Cancer among Scandinavian women with cosmetic breast implants: a pooled long-term follow-up study. Int J Cancer 2009;124:490-493.
100. Lipworth L, Bender TJ, Rossi M, Bosetti C, Negri E, Talamini R, Giacosa A, Franceschi S, McLaughlin JK, La Vecchia C. Dietary vitamin D intake and cancers of the colon and rectum: a case-control study in Italy. Nutr Cancer 2009;61:70-75.
101. Lipworth L, Tarone RE, McLaughlin JK. Breast implants and lymphoma risk: A review of the epidemiologic evidence through 2008. Plast Reconstr Surg 2009;123:790-793.
102. Rossi M, McLaughlin JK, Lagiou P, Bosetti C, Talamini R, Lipworth L, Giacosa A, Montella M, Franceschi S, Negri E, La Vecchia C. Vitamin D intake and breast cancer risk: a case-control study in Italy. Ann Oncol 2009;20:374-378.
103. Lipworth L, Tarone RE, Lund L, McLaughlin JK. Epidemiologic characteristics and risk factors for renal cell cancer. Clin Epidemiol 2009;1:33-43.
104. Fei C, McLaughlin JK, Lipworth L, Olsen J. Maternal levels of perfluorinated chemicals and subfecundity. Human Reproduction 2009; 24:1200-1205.
105. Lagiou P, Hsieh CC, Lipworth L, Samoli E, Okulicz W, Triosi R, Xu B, Hall P, Ekblom A, Adami HO, Trichopoulos D. Insulin-like growth factor levels in cord blood, birth weight and breast cancer risk. Br J Cancer 2009; 100:1794-1798.
106. Eriksen KT, Sørensen M, McLaughlin JK, Lipworth L, Tjønneland A, Overvad K, Raaschou-Nielsen O. Perfluorooctanoate and perfluorooctanesulfonate plasma levels and risk of cancer in the general Danish population. J Natl Cancer Inst 2009; 101:605-609.
107. Lipworth L, Kjølner K, Hölmich LR, Friis S, Olsen JH, McLaughlin JK. Psychological characteristics of Danish women with cosmetic breast implants. Ann Plast Surg 2009; 63:11-14.
108. Hvilsom GB, Hölmich LR, Henriksen TF, Lipworth L, McLaughlin JK, Friis S. Local complications after cosmetic breast augmentation: Results from the Danish Registry for Plastic Surgery of the Breast. Plast Reconstr Surg 2009;124:919-925.
109. Lipworth L, La Vecchia C, Bosetti C, McLaughlin JK. Occupational exposure to rock wool and glass wool and risk of cancers of the lung and the head and neck: a systematic review and meta-analysis. J Occup Environ Med 2009;51:1075-1087.
110. Boffetta P, McLaughlin JK, Vecchia CL, Tarone RE, Lipworth L, Blot WJ. A further plea for adherence to the principles underlying science in general and the epidemiologic enterprise in particular. Int J Epidemiol 2009;38:678-679.
111. Lipworth L, Rossi M, McLaughlin JK, Negri E, Talamini R, Levi F, Franceschi S, La Vecchia C. Dietary vitamin D and cancers of the oral cavity and esophagus. Ann Oncol 2009;20:1576-1581.
112. Rossi M, Lipworth L, Dal Maso L, Talamini R, Montella M, Polesel J, McLaughlin JK, Parpinel M, Franceschi S, Lagiou P, La Vecchia C. Dietary glycemic load and hepatocellular carcinoma with or without chronic hepatitis infection. Ann Oncol 2009;20:1736-1740.

113. Rossi M, Lipworth L, Polesel J, Negri E, Bosetti C, Talamini R, McLaughlin JK, La Vecchia C. Dietary glycemic index and glycemic load and risk of pancreatic cancer. Ann Epidemiol 2010;20:460-465.
114. Lipworth L, McLaughlin JK. Excess suicide risk and other unnatural causes of death among women with cosmetic breast implants: a neglected research priority. Curr Psych Reports 2010;12:234-238.
115. Pira E, Piolatto G, Negri E, Romano C, Boffetta P, Lipworth L, McLaughlin JK, La Vecchia C. Bladder cancer mortality of workers exposed to aromatic amines: a 58-year follow-up. J Natl Cancer Inst 2010;102:1-4.
116. Fei C, McLaughlin JK, Lipworth L, Olsen J. Maternal concentrations of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) and duration of breastfeeding. Scand J Work Environ Health 2010;36:413-421.
117. Hvilsum GB, Hölmich LR, Henriksen TF, Lipworth L, McLaughlin JK, Friis S. Local complications after cosmetic breast augmentation: Results from the Danish Registry for Plastic Surgery of the Breast. Plast Surg Nurs 2010;30:172-179.
118. Fei C, McLaughlin JK, Lipworth L, Olsen J. Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. Environ Res 110:773-777, 2010.
119. Tarone RE, Lipworth L, McLaughlin JK. The epidemiology of environmental perchlorate exposure and thyroid function: a comprehensive review. J Occup Environ Med 2010;52:653-660.
120. Lipworth L, Zucchetto A, Bosetti C, Franceschi S, Talamini R, Serraino D, McLaughlin JK, La Vecchia C, Negri E. Diabetes mellitus and other medical conditions and pancreatic cancer: a case-control study. Diabetes Metab Res Rev 2011;27:255-261.
121. Lucenteforte E, Zucchetto A, Bosetti C, Talamini R, Negri E, Serraino D, Franceschi S, Lipworth L, La Vecchia C. Reproductive and hormonal factors and pancreatic cancer risk in women. Pancreas 2011; 40:460-463.
122. Lagiou P, Samoli E, Lipworth L, Lagiou A, Fang F, Rossi M, Xu B, Yu GP, Adami HO, Hsieh CC, Trichopoulos D. Energy intake during pregnancy in relation to offspring gender by maternal height. Eur J Epidemiol 2011;26:39-44.
123. Lagiou P, Samoli E, Okulicz W, Xu B, Lagiou A, Lipworth L, Georgila C, Vatten L, Adami HO, Trichopoulos D, Hsieh CC. Maternal and cord blood hormone levels in the United States and China and the intrauterine origin of breast cancer. Ann Oncol 2011;22:1102-1108.
124. Lipworth L, Holmich LR, McLaughlin JK. Silicone breast implants and connective tissue disease: no association. Semin Immunopathol 2011;33:287-294.
125. McLaughlin JK, Mumma MT, Sonderman JS, Farnsworth EP, Lipworth L. Cancer mortality among workers at a satellite manufacturing facility . J Occup Environ Med 2011;53:427-433.
126. Pelucchi C, Bosetti C, Negri E, Lipworth L, La Vecchia C. Olive oil and cancer risk: an update of epidemiological findings to 2010. Curr Pharm Des 2011;17:805-812.
127. Lipworth L, Tarone RE, McLaughlin JK. Renal cell cancer among African Americans: an epidemiologic review. BMC Cancer 2011;11:133.
128. Lipworth L, McLaughlin JK, Tarone RE, Blot WJ. Renal cancer paradox: higher incidence but not higher mortality among African-Americans. Eur J Cancer Prev 2011;20:331-333.

129. Lipworth L, Sonderman JS, Mumma MT, Tarone RE, Marano DE, Boice JD Jr, McLaughlin JK. Cancer mortality among aircraft manufacturing workers: an extended follow-up. *J Occup Environ Med* 2011;53:992-1007.
130. Hvilsom GB, Friis S, Frederiksen K, Steding-Jessen M, Henriksen TF, Lipworth L, McLaughlin JK, Elberg JJ, Damsgaard TE, Hölmich LR. The clinical course of immediate breast implant reconstruction after breast cancer. *Acta Oncol* 2011;50:1045-1052.
131. Hvilsom GB, Hölmich LR, Steding-Jessen M, Frederiksen K, Henriksen TF, Lipworth L, McLaughlin JK, Elberg JJ, Damsgaard TE, Friis S. Delayed breast implant reconstruction: a 10-year prospective study. *J Plast Reconstr Aesthet Surg* 2011;64:1466-1474.
132. Hvilsom GB, Holmich LR, Steding-Jessen M, Frederiksen K, Henriksen TF, Lipworth L, McLaughlin JK, Elberg JJ, Damsgaard TE, Friis S. Delayed breast implant reconstruction: is radiation therapy associated with capsular contracture or reoperations? *Ann Plast Surg* 2012;68:246-252.
133. Lipworth L, Sonderman JS, Tarone RE, McLaughlin JK. Review of epidemiologic studies of dietary acrylamide intake and risk of cancer. *Eur J Cancer Prev* 2012;21:375-386.
134. McLaughlin JK, Sonderman JS, Tarone RE, Mumma MT, Lipworth L. Cancer incidence among workers at a satellite manufacturing facility. *J Occup Environ Med* 2012;54:1500-1505.
135. Lipworth L, Okafor H, Mumma MT, Edwards TL, Roden DM, Blot WJ, Darbar D. Race-specific impact of atrial fibrillation risk factors in blacks and whites in the Southern Community Cohort Study. *Am J Cardiol* 2012;110:1637-1642.
136. Lipworth L, Mumma MT, Cavanaugh KL, Edwards TL, Ikizler TA, Tarone RE, McLaughlin JK, Blot WJ. Incidence and predictors of end stage renal disease among low-income blacks and whites. *PLoS One* 2012;7:e48407.
137. Lipworth L, Sonderman JS, Tarone RE, McLaughlin JK. Acrylamide: a human cancer risk? *Eur J Cancer Prev* 2013;22:193-194.
138. Eriksen KT, Raaschou-Nielsen O, McLaughlin JK, Lipworth L, Tjønneland A, Overvad K, Sørensen M. Association between plasma PFOA and PFOS levels and total cholesterol in a middle aged Danish population. *PLoS One* 2013;8:e56969 [Epub ahead of print].
139. Cohen SS, Matthews CE, Bradshaw PT, Lipworth L, Buchowski MS, Signorello LB, Blot WJ. Sedentary behavior, physical activity, and likelihood of breast cancer among black and white women: a report from the Southern Community Cohort Study. *Cancer Prev Res* 2013;6:566-576.
140. Sanderson N, Fowke J, Lipworth L, Han X, Ukoli F, Coker AL, Blot WJ, Hargreaves MK. Diabetes and prostate cancer screening in black and white men. *Cancer Causes Control* 2013;24:1893-1899.
141. Lipworth L, Fazio S, Kabagambe EK, Munro H, Nwazue VC, Tarone RE, McLaughlin JK, Blot WJ, Sampson UKA. A prospective study of statin use and mortality among 67,385 Blacks And Whites In The Southeastern United States. *Clin Epidemiol* 2013;6:15-25.
142. Sampson UKA, Edwards TL, Jahangir E, Munro H, Wariboko M, Wassef MG, Fazio S, Mensah GA, Kabagambe EK, Blot WJ, Lipworth L. Factors associated with the prevalence of hypertension in the southeastern United States: Insights from 69, 211 blacks and whites in the Southern Community Cohort Study. *Circ Cardiovasc Qual Outcomes* 2014;7:33-54.

- **Selected as Vanderbilt Epidemiology Center Top 10 Paper of 2013**

143. Cui Y, Deming-Halverson S, Beeghly-Fadiel A, Lipworth L, Shrubsole MJ, Fair AM, Shu XO, Zheng W. Interactions of hormone replacement therapy, body weight and bilateral oophorectomy in breast cancer risk. Clin Cancer Res 2014;20:1169-1178.
144. Kiage JN, Merrill PD, Judd SE, He K, Lipworth L, Cushman M, Howard VJ, Kabagambe EK. Intake of trans fat and incidence of stroke in the REasons for Geographic And Racial Differences in Stroke (REGARDS) cohort. Am J Clin Nutr 2014;99:1071-1076.
145. Sanderson M, Lipworth L, Han X, Beeghly-Fadiel A, Shen-Miller D, Patel K, Blot WJ, Hargreaves MK. Mammography use among women with and without diabetes: results from the Southern Community Cohort Study. J Epidemiol Glob Health 2014;4:223-230.
146. Jahangir E, Lipworth L, Edwards TL, Kabagambe EK, Mumma MT, Mensah GA, Fazio S, Blot WJ, Sampson UKA. Smoking, sex, risk factors and abdominal aortic aneurysms: a prospective study of 18 782 persons aged above 65 years in the Southern Community Cohort Study. J Epidemiol Community Health 2015;69:481-488.
147. Sanderson M, Lipworth L, Shen-Miller D, Nechuta S, Beeghly-Fadiel A, Shrubsole MJ, Zheng W. Energy-related indicators and breast cancer risk among white and black women. PLoS One 2015;10:e0125058.
148. Lipworth L, Morgans AK, Edwards TL, Barocas DA, Chang SS, Herrell SD, Penson DF, Resnick MJ, Smith JA, Clark PE. Renal cell cancer histologic subtype distribution differs by race and sex. BJU Int 2016;117:260-265.
149. Shen T, Shu XO, Xiang YB, Li HL, Cai H, Gao YT, Zheng W, Lipworth L. Association of hypertension and obesity with renal cell carcinoma risk: a report from the Shanghai Men's and Women's Health Studies. Cancer Causes Control 2015;26:1173-1180.
150. Kantor ED, Lipworth L, Fowke JH, Giovannucci EL, Mucci LA, Signorello LB. Statin use and risk of prostate cancer: Results from the Southern Community Cohort Study. Prostate 2015;75:1384-1393.
151. Hedgeman E, Lipworth L, Lowe K, Saran R, Do T, Fryzek J. International burden of chronic kidney disease and secondary hyperparathyroidism: a systematic review of the literature and available data. Int J Nephrol 2015;2015:184321.
152. Glenn KR, Slaughter JC, Fowke JH, Buchowski MS, Matthews CE, Signorello LB, Blot WJ, Lipworth L. Physical activity, sedentary behavior and all-cause mortality among blacks and whites with diabetes. Am J Epidemiol 2015;25:649-655.
153. Kiage JN, Sampson UK, Lipworth L, Fazio S, Mensah GA, Yu Q, Munro H, Akwo EA, Dai Q, Blot WJ, Kabagambe EK. Intake of polyunsaturated fat in relation to mortality among statin users and non-users in the Southern Community Cohort Study. Nutr Metab Cardiovasc Dis 2015;25:1016-1024.
154. Akwo EA, Cavanaugh KL, Ikizler TA, Blot WJ, Lipworth L. Increased body mass index may be associated with greater risk of end-stage renal disease in whites compared to blacks; a nested case-control study. BMC Nutrition 2015;1:24.
155. Setiawan VW, Lim U, Lipworth L, Lu SC, Shepherd J, Ernst T, Wilkens LR, Henderson BE, Le Marchand L. Sex and ethnic differences in the association of obesity with risk of hepatocellular carcinoma. Clin Gastroenterol Hepatol 2016;14:309-316.

156. Malhotra R, Cavanaugh KL, Blot WJ, Ikizler TA, Lipworth L*, Kabagambe EK*. Dietary polyunsaturated fatty acids and incidence of end-stage renal disease in the Southern Community Cohort Study. BMC Nephrol 2016;17:152.
157. Malhotra R, Cavanaugh KL, Blot WJ, Ikizler TA, Lipworth L*, Kabagambe EK*. Higher protein intake is associated with increased risk for end-stage renal disease among blacks with diabetes in the Southern Community Cohort Study. Nutr Metab Cardiovasc Dis 2016 [Epub ahead of print].
158. Kensinger C, Bian A, Fairchild M, Chen G, Lipworth L, Ikizler TA, Birdwell K. Long term evolution of endothelial function during kidney transplantation. BMC Nephrol 2016;17:160.
159. Kensinger C, Hernandez A, Bian A, Fairchild M, Chen G, Lipworth L, Ikizler TA, Birdwell K. Longitudinal assessment of cardiac morphology and function following kidney transplantation. Clin Transplant Epub 2016 Nov 24.
160. Lipworth L, Abdel-Kader K, Morse J, Stewart TG, Kabagambe EK, Parr S, Birdwell KA, Matheny ME, Hung AM, Blot WJ, Ikizler TA, Siew ED. High prevalence of non-steroidal anti-inflammatory drug use among acute kidney injury survivors in the Southern Community Cohort Study. BMC Nephrol 2016;17:189.
161. Phelan CM, Kuchenbaecker KB, Tyrer RP, [et al, including Lipworth L]. Identification of 12 new susceptibility loci for different histotypes of epithelial ovarian cancer. Nat Genet 2017;49:680-691.
162. Akwo EA, Kabagambe EK, Wang TJ, Harrell FE Jr, Blot WJ, Mumma M, Gupta DK*, Lipworth L*. Heart failure incidence and mortality in the Southern Community Cohort Study. Circ Heart Fail 2017;10:e003553.
163. Scelo G, Purdue MP, Brown KM, [et al, including Lipworth L]. Genome-wide association study identifies multiple risk loci for renal cell carcinoma. Nat Commun 2017;8:15724.
164. Bejan CA, Angiolillo J, Conway D, Nash R, Shirey-Rice JK, Lipworth L, Cronin RM, Pulley J, Kripalani S, Barkin S, Johnson KB, Denny JC. Mining 100 million clinical notes to find homelessness and adverse childhood experiences: 2 case studies of rare and severe social determinants of health in electronic health records. JAMIA 2018;25:61-71.
165. Bachmann JM, Huang S, Gupta D, Lipworth L, Mumma M, Blot WJ, Akwo EA, Kripalani S, Whooley M, Wang TJ, Freiberg MS. Association of neighborhood socioeconomic context with participation in cardiac rehabilitation. JAHA 2017;6:e006260.
166. Machiela MJ, Hoffman JN, Carreras-Torres R, [et al, including Lipworth L]. Genetic Variants Related to Longer Telomere Length are Associated with Increased Risk of Renal Cell Carcinoma. Eur Urol 2017;72:747-754.
167. Salat H, Javier A, Siew ED, Figueroa R, Lipworth L, Kabagambe E, Bian A, Stewart TG, El-Sourady MH, Karlekar M, Cardona CY, Ikizler TA, Abdel-Kader K. Nephrology provider prognostic perceptions and care delivered to older adults with advanced kidney disease. Clin J Am Soc Nephrol 2017;12:1762-1770.
168. Shu X, Cai H, Xiang YB, Li H, Lipworth L, Miller N, Zheng W, Shu XO, Hsi RS. Nephrolithiasis among middle aged and elderly urban Chinese: a report from prospective studies in Shanghai. J Endourol 2017;31:1327-1334.

169. Kabagambe EA, Lipworth L, Labadie RF, Hood LJ, Francis DO. Erythrocyte folate, serum vitamin B12, and hearing loss in the 2003-2004 National Health and Nutrition Examination Survey (NHANES). Eur J Clin Nutr 2018 [Epub ahead of print].
170. Akwo EA, Kabagambe EK, Harrell FE, Blot WJ, Bachmann JM, Wang TJ, Gupta DK*, Lipworth L*. Neighborhood deprivation predicts heart failure risk in a low-income population of blacks and whites in the southeastern United States. Circ Cardiovasc Qual Outcomes 2018;11:e004052.
 - **Selected for AHA Press release.** AHA Science Media Relations estimates an audience of at least 115 million people viewed, read or heard this news. In addition, it reached 68,610 people via AHA social media channels.
 - **Selected as Vanderbilt Epidemiology Center Top 10 Paper of 2017**
 - Selected Media Links:
 - <https://news.vanderbilt.edu/2018/01/09/heart-failure-risk-predicted-by-communities-not-wealth/>
 - <https://newsroom.heart.org/news/neighborhood-factors-may-predict-heart-failure>
 - <https://health.usnews.com/health-care/articles/2018-01-09/life-in-poor-neighborhoods-is-hard-on-the-heart>
 - <https://www.drugs.com/news/poor-neighborhoods-hard-heart-68312.html>
 - <http://www.health.com/healthday/life-poor-neighborhoods-hard-heart>
 - <https://www.sciencedaily.com/releases/2018/01/180109090236.htm>
 - <https://www.medicalnewstoday.com/articles/320571.php>
 - <https://www.healio.com/cardiology/hf-transplantation/news/online/%7bed021601-ef1b-4ae9-8f79-188c19023727%7d/neighborhood-deprivation-increases-risk-for-hf>
171. Malhotra R, Lipworth L, Cavanaugh KL, Young BA, Tucker KL, Carithers TC, Taylor HA, Correa A, Kabagambe EK, Ikizler TA. Protein intake and long-term change in glomerular filtration rate in the Jackson Heart Study. J Ren Nutr 2018;28:245-250.
172. Hsi RS, Kabagambe EK, Shu X, Han X, Miller NL, Lipworth L. Race- and sex-related differences in nephrolithiasis risk among blacks and whites in the Southern Community Cohort Study. Urology 2018;118:36-42.
173. Shu X, Cai H, Xiang YB, Li H, Lipworth L, Miller NL, Zheng W, Shu XO, Hsi RS. Green tea intake and risk of incident kidney stones: Prospective cohort studies in middle-aged and elderly Chinese individuals. Int J Urol 2018 [Epub ahead of print].
174. Ramer SJ, McCall NN, Robinson-Cohen C, Siew ED, Salat H, Bian A, Stewart TG, El-Sourady MH, Karlekar M, Lipworth L, Ikizler TA, Abdel-Kader K. Health Outcome Priorities of Older Adults with Advanced CKD and Concordance with Their Nephrology Providers' Perceptions. J Am Soc Nephrol 2018;29:2870-2878.
175. Johansson M, Carreras-Torres R, Scelo G, [et al, including Lipworth L]. The influence of obesity-related factors in the etiology of renal cell carcinoma – A mendelian randomization study. PLoS Med 2019;16:e1002724.
176. Suh M, Wikoff D, Lipworth L, Goodman M, Fitch S, Mittal L, Ring C, Proctor D. Hexavalent chromium and stomach cancer: a systematic review and meta-analysis. Crit Rev Toxicol 2019;21:1-20.
177. Pike M, Stewart TG, Morse J, Ormsby P, Siew ED, Hung A, Abdel-Kader K, Ikizler TA, Lipworth L, Robinson-Cohen C. APOL1, acid load, and CKD progression. Kidney Int Rep 2019;4:946-954.

178. Sanderson M, Lipworth L, Shrubsole MJ, Andersen SW, Shu XO, Zheng W, Hargreaves MK, Blot WJ. Diabetes, obesity, and subsequent risk of postmenopausal breast cancer among white and black women in the Southern Community Cohort Study. Cancer Causes Control 2019;30:425-433.
179. Laskar RS, Muller DC, Li P, [et al, including Lipworth L]. Sex specific associations in genome wide association analysis of renal cell carcinoma. Eur J Hum Genet 2019 [Epub ahead of print].
180. Baddour NA, Robinson-Cohen C, Lipworth L, Bian A, Stewart TG, Jhamb M, Siew ED, Abdel-Kader K. The surprise question and self-rated health are useful screens for frailty and disability in older adults with chronic kidney disease. J Palliat Med 2019 [Epub ahead of print].
181. Bock J, Stewart TG, Robinson-Cohen C, Morse J, Kabagambe EK, Cavanaugh KL, Birdwell KA, Hung AM, Abdel-Kader K, Siew ED, Akwo EA, Blot WJ, Ikizler TA, Lipworth L. Racial disparities in end-stage renal disease in a high-risk population: the Southern Community Cohort Study. BMC Nephrol 2019;20:308.
182. Schildkraut JM, Peres LC, Bethea TN, Camacho F, Chyn D, Cloyd EK, Bandera EV, Beeghly-Fadiel A, Lipworth L, Joslin CE, Davis FG, Moorman PG, Myers E, Ochs-Balcom HM, Setiawan VW, Pike MC, Wu AH, Rosenberg L. Ovarian Cancer in Women of African Ancestry (OCWAA) consortium: a resource of harmonized data from eight epidemiologic studies of African American and white women. Cancer Causes Control 2019;30:967-978.
183. Pike M, Taylor J, Kabagambe E, Stewart TG, Robinson-Cohen C, Morse J, Akwo E, Abdel-Kader K, Siew ED, Blot WJ, Ikizler TA, Lipworth L. The association of exercise and sedentary behaviours with incident end-stage renal disease: the Southern Community Cohort Study. BMJ Open 2019;9:e030661
184. Baddour NA, Siew ED, Robinson-Cohen C, Salat H, Mason OJ, Stewart TG, Karlekar M, El-Sourady MH, Lipworth L, Abdel-Kader K. Serious illness treatment preferences for older adults with advanced CKD. J Am Soc Nephrol 2019;30:2252-2261.
185. Kubicki DM, Xu M, Adwo EA, Dixon D, Munoz D, Blot WJ, Wang TJ, Lipworth L*, Gupta DK*. Race and sex differences in modifiable risk factors and incident heart failure. J Am Coll Cardiol: Heart Failure in press.

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Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/loren.lipworth.1/bibliography/public>

Book chapters

1. Trichopoulos D, Lipworth L. The role of diet in the etiology of breast cancer. In: Austrian Nutrition Society, Widhalm K, Leibetseder J, Bauernfried M, editors. Over- and undernutrition in Europe. Proceedings of the Seventh European Nutrition Conference, Vienna, Hofburg, May 24-28, 1995.
2. Trichopoulos D, Lipworth L, Petridou E, Adami H-O. Epidemiology of cancer. In: DeVita VT Jr, Hellman S, Rosenberg SA, editors. Cancer: principles and practice of oncology. 5th edition. Philadelphia: J.B. Lippincott Co., 1997.

3. Lipworth L. Review of the epidemiology of breast cancer. In: Ioannidou-Mouzaka L, editor. Synchronous Mastologia. Athens: 1996, pp. 425-450. (in Greek).
4. Blot WJ, Lipworth L, McLaughlin JK. Esophageal cancer: epidemiology and risk factors. In: Kelsen DP, Daly J, Kern S, Levin B, Tepper J, editors. Gastrointestinal oncology: Principles and practice. Philadelphia: Lippincott Williams & Wilkins, 2002, pp. 203-209.
5. McLaughlin JK, Lipworth L. Epidemiology and biostatistics. In: McCunney RJ (Ed): A Practical Approach to Occupational and Environmental Medicine, 3rd Ed. Philadelphia, PA: Lippincott Williams and Wilkins, pp 571-581, 2003.
6. McLaughlin JK, Lipworth L, Tarone RE, Blot WJ. Renal cancer. In: Schottenfeld D and Fraumeni JF Jr (Eds). Cancer Epidemiology and Prevention, Third Edition. New York: Oxford University Press, 2006, pp 1087-1100.
7. Lipworth L, McLaughlin JK. The safety of breast implants: epidemiologic studies. In: Peters W, Brandon H, Jerina KL, Wolf C, Young VL, editors. Biomaterials in plastic surgery: Breast implants. Oxford: Woodhead Publishing Limited, 2012, pp 121-153.
8. Cohen SS, Lipworth L. Role of ethnic differences in mediators of energy balance. In: Berger NL, editor. Impact of Energy Balance on Cancer Disparities. Switzerland: Springer International Publishing, 2014.

Letters to the Editor

1. Lipworth L, Trichopoulos D, Petridou E. Maternal pregnancy estrogens, breast cancer risk, and the Utah data (letter). J Natl Cancer Inst 1995;87:144-145.
2. Lipworth L, Trichopoulos D, Adami H-O, Ekblom A. Weight gain in infancy and cancer of the ovary (letter). Lancet 1995;345:1515.
3. Tarone RE, Lipworth L, McLaughlin JK. Re: Serious gastrointestinal events from low dose analgesic use. J Rheumatol 2004;31:1008-1009.
4. Lipworth L, Tarone RE, McLaughlin JK. Reply to S. A. Fayer and Z. P. Lorenc Letter to the Editor. Ann Plast Surg 2007;59:732-733.
5. Lipworth L, Laggiou P, Hsieh C-C, Trichopoulos D. Re: Height as an explanatory factor for sex differences in human cancer. J Natl Cancer Inst 2013;105:1762.
6. Kiage JN, Sampson UK, Lipworth L, Fazio S, Mensah GA, Yu Q, Munro H, Akwo EA, Dai Q, Blot WJ, Kabagambe EK. Polyunsaturated fat intake and mortality in non-statin users, is there an independent relationship? The authors reply. Nutr Metab Cardiovasc Dis 2016;26:78-79.

Abstracts (selected)

1. Jahangir E, **Lipworth L**, Mumma MT, Edwards TL, Blot WJ, Sampson UKA. Predictors of incident abdominal aortic aneurysm in the elderly: insights from the Southern Community Cohort Study. American Heart Association 2012 Scientific Session.
2. Sampson UKA, Edwards TL, Jahangir E, Munro H, Wariboko M, Wassef MG, Fazio S, Mensah GA, Kabagambe EK, Blot WJ, **Lipworth L**. Racial Differences in the Prevalence of Uncontrolled and Unreported Hypertension in the Southeastern United States: Insights from almost 70,000 Blacks

and Whites in the Southern Community Cohort Study. American College of Cardiology 2013 Scientific Session.

3. Kiage JN, Merrill PD, Judd SW, He K, **Lipworth L**, Cushman M, Howard VJ, Kabagambe EK. *Trans-fat intake and incidence of stroke in the REasons for Geographical and Racial Differences in Stroke (REGARDS) Cohort.* American Heart Association 2013 Scientific Session.
4. Glenn KR, Slaughter JC, Fowke JH, Signorello LB, Blot WJ, **Lipworth L**. Physical activity, sedentary behavior and all-cause mortality among blacks and whites with diabetes. American Heart Association 2014 Fellows Research Day.
5. Kiage JN, Sampson UKA, **Lipworth L**, Fazio F, Yu Q, Munro H, Akwo EA, Blot WJ, Kabagambe EK. Association between polyunsaturated fat consumption and hypertension among statin users and non-users. American Heart Association Epidemiology and Prevention/Nutrition, Physical Activity and Metabolism 2014 Scientific Session.
6. Akwo EA, Cavanaugh KL, Ikizler TA, Blot WJ, **Lipworth L**. The Association between Body Mass Index and Incident End-Stage Renal Disease in Blacks and Whites: The Southern Community Cohort Study. Society for Epidemiologic Research 2014 Annual Meeting.
7. Sanderson M, **Lipworth L**, Shen-Miller D, Williams K, Zheng W, Hargreaves M, Blot WJ. Interaction of diabetes and obesity in postmenopausal breast cancer risk among white and black women in the Southern Community Cohort Study. Society for Epidemiologic Research 2014 Annual Meeting.
8. Shen T, Shu XO, Xiang YB, Cai H, Li HL, Gao YT, Zheng W, **Lipworth L**. Association of hypertension and obesity with renal cell carcinoma risk: a report from the shanghai men's and women's health studies. Society for Epidemiologic Research 2014 Annual Meeting.
9. Kantor ED, **Lipworth L**, Fowke JH, Mucci LA, Signorello LB. Statin Use and Risk of Prostate Cancer: Results from the Southern Community Cohort Study. Society for Epidemiologic Research 2014 Annual Meeting.
10. Setiawan VW, **Lipworth L**, Wilkens LR, Le Marchand L, Blot WJ, Henderson BE. Racial/ethnic differences in the association of obesity with hepatocellular carcinoma risk. AACR Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved 2014.
11. Sanderson M, **Lipworth L**, Shen-Miller D, Nechuta S, Shrubsole M, Beeghly-Fadiel A, Shu XO, Zheng W. Interaction of diabetes and obesity in postmenopausal breast cancer risk among black women. AACR Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved 2015.
12. Akwo EA, Kabagambe ED, Wang TJ, Harrell FE, Blot WJ, Gupta DK, **Lipworth L**. Heart failure incidence and mortality in the Southern Community Cohort Study. American Heart Association EPI/Lifestyle 2015 Scientific Session.
13. Kabagambe EK, Kiage JN, Judd SE, Slaughter JC, **Lipworth L**, Sampson U, Djousse L, Rimm EB, Cushman M, Fazio S, Safford M, Howard G. Alcohol consumption, statin use and risk of all-cause mortality in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Cohort. American Heart Association EPI/Lifestyle 2015 Scientific Session.
14. James LO, Kiage JN, **Lipworth L**, Sampson U, Kabagambe EK. Alcohol Consumption, Transferrin Saturation and Risk of All-Cause Mortality in The National Health and Nutrition Examination Surveys. American Heart Association EPI/Lifestyle 2015 Scientific Session.

15. Cohen SS, Kabagambe EK, Shirey-Rice J, Hardin J, Monda K, Fryzek JP, Fazio W, Denny JC, Wei WQ, **Lipworth L**. Achievement of LDL-cholesterol reduction targets is associated with reduced cardiovascular disease risk among patients with familial hypercholesterolemia in a large electronic medical record database. American Heart Association 2015 Scientific Session.
16. Cohen SS, Shirey-Rice J, Hardin J, Monda K, Fryzek JP, Fazio S, Denny JC, Wei WQ, Lipworth L. Identification of patients with familial hypercholesterolemia using the Dutch Lipid Network criteria in Electronic Health Records. American Heart Association EPI/Lifestyle 2016 Scientific Session.
17. **Lipworth L**, Shirey-Rice J, Wei WQ, Hardin J, Shi G, Monda K, Fryzek JP, Fazio S, Cohen SS, Denny JC. Identification and characterization of heterozygous familial hypercholesterolemia patients using the Vanderbilt University Medical Center Synthetic Derivative database. European Society of Cardiology 2015 Conference.
18. Singh J, Kabagambe EK, Xu M, Gaziano TA, Wang TJ, Blot WJ, Gupta DK, **Lipworth L**. Evaluation of a non-laboratory-based cardiovascular disease (CVD) risk score in predicting CVD mortality in the Southern Community Cohort Study. American Heart Association 2015 Scientific Sessions.
19. Malhotra R, Cavanaugh KL, Blot WJ, Ikizler TA, **Lipworth L**, Kabagambe EK. High protein intake is associated with increased risk for incident end-stage renal disease among blacks in the Southern Community Cohort Study. American Society of Nephrology 2015 Kidney Week.
20. Gregg R, Glaser Z, Emeruwa C, Wong J, Johnson C, Holmes A, Ellis D, **Lipworth L**, Edwards T, Clark PE. Association of single nucleotide polymorphisms with renal cell carcinoma metastases in patients with dual diagnoses of renal cell carcinoma and melanoma. Society of Urologic Oncology 2016 Annual Meeting.
21. Cavanaugh KL, Kabagambe EK, Morse J, Stewart TG, Bowman AB, Zhang Y, Blot WJ, Ikizler TA, **Lipworth L**. Arsenic exposure and incident ESRD in the Southern Community Cohort Study (SCCS). American Society of Nephrology 2017 Kidney Week.
22. Bock F, Stewart TG, Morse J, Kabagambe EK, Cavanaugh KL, Siew ED, Mumma MT, Blot WJ, Ikizler TA, **Lipworth L**. The Southern Community Cohort Study (SCCS): a population based resource for research in CKD. American Society of Nephrology 2017 Kidney Week.
23. Taylor JM, Kabagambe EK, Stewart TG, Morse J, Siew ED, Blot WJ, **Lipworth L**, Ikizler TA. Daily sedentary time and physical activity are independently associated with ESRD risk. American Society of Nephrology 2017 Kidney Week.
24. Bock F, Siew ED, Morse J, Stewart TG, Blot WJ, Ikizler TA, **Lipworth L**. Non-steroidal anti-inflammatory drug (NSAID) drug use and end stage renal disease (ESRD) in the Southern Community Cohort Study (SCCS). American Society of Nephrology 2017 Kidney Week.

EXHIBIT B

Review of epidemiologic studies of dietary acrylamide intake and the risk of cancer

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Conjectured associations between dietary acrylamide intake and cancer have been evaluated in more than 15 epidemiologic studies examining almost every major cancer site. We have critically reviewed the epidemiologic studies of estimated dietary acrylamide exposure and cancer. As substantially greater acrylamide exposure occurs through tobacco smoke than dietary exposure, we present the results separately for never smokers or adjusted statistically for smoking status, where possible. After an extensive examination of the published literature, we found no consistent or credible evidence that dietary acrylamide increases the risk of any type of cancer in humans, either overall or among nonsmokers. In particular, the collective evidence suggests that a high level of dietary acrylamide intake is not a risk factor for breast, endometrial, or ovarian cancers, which have generated particular interest because of a conjectured hormonal mechanism of acrylamide. Moreover, the absence of a positive association between smoking and ovarian and endometrial cancers suggests that any association of these cancers with the much lower, more sporadic dietary acrylamide intake is unlikely. In conclusion, epidemiologic

studies of dietary acrylamide intake have failed to demonstrate an increased risk of cancer. In fact, the sporadically and slightly increased and decreased risk ratios reported in more than two dozen papers examined in this review strongly suggest the pattern one would expect to find for a true null association over the course of a series of trials. Therefore, continued epidemiologic investigation of acrylamide and cancer risk appears to be a misguided research priority. *European Journal of Cancer Prevention* 21:375–386 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

As a result of animal studies showing acrylamide to be a multiorgan carcinogen in rodents (Bull *et al.*, 1984; Johnson *et al.*, 1986), early concerns were raised about the health of workers with potential occupational exposure to acrylamide. In 1994, acrylamide was classified by the International Agency for Research on Cancer (IARC) as ‘probably carcinogenic to humans’ (IARC Group 2A) on the basis of positive evidence from animal studies and ‘inadequate’ epidemiologic evidence (International Agency for Research on Cancer, 1994).

The association between acrylamide exposure in occupational settings and the occurrence of cancer has been studied extensively with long-term follow-up in three occupational cohorts of acrylamide-exposed workers (Sobel *et al.*, 1986; Collins *et al.*, 1989; Marsh *et al.*, 1999, 2007; Swaen *et al.*, 2007). These studies have shown no increased mortality from cancer overall or from specific types of cancer, nor do they support a positive dose–response relation on the basis of cumulative exposure,

duration of exposure, or other exposure metrics, indicating that acrylamide is not an occupational carcinogen (Pelucchi *et al.*, 2011b).

About 10 years ago, Swedish investigators reported that acrylamide was formed during high-temperature cooking of animal feed and during frying, baking, and roasting of numerous common foods (Tareke *et al.*, 2000; Svensson *et al.*, 2003). Since then, hypothesized associations between dietary acrylamide intake and cancer have been evaluated in a large number of studies in Sweden, other European countries, and the USA, examining almost every major cancer site. Here, we present a critical, qualitative review of the epidemiologic studies of dietary acrylamide intake and cancer.

Methods

We searched the PubMed database using the terms ‘acrylamide’ AND ‘cancer’ AND ‘epidemiology OR case-control OR cohort.’ Manual searches using the PubMed related citation function extended the number of references identified, and additional references were ascertained by cross-checking the reference lists of the identified publications, including reviews and

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Website (www.eurjcancerprev.com).

meta-analyses. We did not include papers evaluating associations with single acrylamide-containing foods such as fried potatoes, as the intake of any individual food will fail to capture a significant portion of dietary acrylamide consumption. Moreover, a comprehensive review of such studies concluded that they provide no consistent evidence of carcinogenic potential (Pelucchi *et al.*, 2011b). The relevant published analytic epidemiologic studies were evaluated by cancer site, and individual results are summarized in Table 1. Additional descriptions and details about individual studies are presented in an online supplementary table (<http://www.iei.us/Acrylamide-Supplementary-Table.pdf>). On the basis of the published studies, a qualitative summary of the results is presented in the text. When presenting relative risk (RR) estimates, the highest level of acrylamide consumption is compared with the lowest consumption level, usually in quintile or quartile levels.

Results

Almost all studies estimated dietary acrylamide exposure using a detailed self-reported food frequency questionnaire (FFQ), along with the computation of intake using national and international databases of the mean or the median acrylamide content in individual foods. In some studies, the accuracy of an FFQ instrument has been evaluated using self-reported dietary records in a sample of the study population by correlating the major acrylamide food sources from the two dietary assessment methods. However, the validity of the reported quantitative estimates of total dietary acrylamide intake has never been assessed directly. It has been argued that using an FFQ along with the average values reported in national databases reliably ranks individuals into high or low acrylamide intake categories, even if estimated amounts of acrylamide intake are less precise and large variations in acrylamide concentrations within individual foods exist by brand and method of preparation (Burley *et al.*, 2010; Konings *et al.*, 2010).

It should be noted that substantially greater acrylamide exposure occurs through tobacco smoke than dietary exposure, and levels of acrylamide-hemoglobin and glycidamide-hemoglobin adducts (markers of internal acrylamide dose) have been shown to be markedly higher among smokers compared with nonsmokers (Schettgen *et al.*, 2004; Hagmar *et al.*, 2005; Bjellaas *et al.*, 2007). Therefore, most epidemiologic studies have attempted to evaluate the relation between dietary acrylamide intake and the risk of cancer restricted to a subgroup of never smokers or adjusted statistically for smoking status, and, when available, we present these results separately in this review.

As the epidemiologic evidence for multiple cancer sites derives for the most part from four large cohort studies, the Netherlands Cohort Study on Diet and Cancer

(NLCS) (Van den Brandt *et al.*, 1990; Hogervorst *et al.*, 2007, 2008a, 2009a; Schouten *et al.*, 2009), the Swedish Mammography Cohort (SMC) (Mucci *et al.*, 2006), and the Nurses' Health Studies (NHS I and II) (Wilson *et al.*, 2009b, 2009c, 2010), the methods of these studies are briefly summarized in an online supplement (<http://www.iei.us/Acrylamide-Supplementary-Table.pdf>).

Breast cancer

The risk of breast cancer in relation to dietary acrylamide intake has been evaluated in six cohort studies in Europe and the USA, and in a case-control study from Italy and Switzerland (Mucci *et al.*, 2005; Pelucchi *et al.*, 2006; Hogervorst *et al.*, 2007; Larsson *et al.*, 2009b; Wilson *et al.*, 2009b, 2010; Burley *et al.*, 2010). A number of studies have evaluated breast cancer separately by tumor estrogen receptor (ER) and progesterone receptor (PR) status, because of a conjectured modulation by acrylamide of sex hormone systems (Hogervorst *et al.*, 2010).

The earliest report came from the Swedish Women's Lifestyle and Health Cohort of 43 404 mostly premenopausal women with almost half a million person-years of follow-up through 2002 (Mucci *et al.*, 2005). An estimated RR of 1.19 [95% confidence interval (CI) 0.91–1.55] was observed for breast cancer associated with the highest quintile of acrylamide intake (mean 44 µg/day) compared with the lowest (12 µg/day). Similarly, in the SMC, 2952 incident cases of breast cancer were diagnosed during a mean follow-up of 17.4 years, and no association was observed for the highest versus the lowest intake of acrylamide (RR = 0.91), either for breast cancer overall or by ER or PR status (Larsson *et al.*, 2009b). Moreover, in stratified analyses, there was no association between intake of acrylamide and breast cancer among never smokers or ever smokers.

In both the NHS I and the NHS II (Wilson *et al.*, 2009b, 2010), there was no evidence that intake of acrylamide was associated with an increased risk of either postmenopausal or premenopausal breast cancer. The RRs for the highest quintile of acrylamide intake were just below 1.0 in both studies, and the results were similar irrespective of smoking status or ER and PR status of the breast tumors.

The UK Women's Cohort study followed 15 951 premenopausal and 17 779 postmenopausal women for a median of 11 years (Burley *et al.*, 2010), during which time 1084 breast cancers were diagnosed in the cohort. Overall, breast cancer was not associated with intake of acrylamide, with an RR of 1.16 (95% CI 0.88–1.52) for the highest quintile, and RRs of 0.98 when restricted to never smokers and 0.97 when restricted to postmenopausal women. Among premenopausal women, the RR for breast cancer was nonsignificantly elevated (RR = 1.47; 95% CI 0.96–2.27) among those in the highest quintile of acrylamide intake, but among never smokers, the RR for the highest quintile was 1.17 (95% CI 0.69–2.00).

Table 1 Summary of the results from epidemiologic studies of dietary acrylamide and cancer

Study	Total population					Never smokers				
	Cases	High vs. low estimated intake		Continuous (per 10 µg/day)		Cases	High vs. low estimated intake		Continuous (per 10 µg/day)	
		RR	95% CI	RR	95% CI		RR	95% CI	RR	95% CI
Breast cancer										
Swedish Women's Lifestyle and Health (Mucci <i>et al.</i> , 2005)	667	1.19	0.91–1.55							
Italy and Switzerland ^a (Pelucchi <i>et al.</i> , 2006)	2900	1.06	0.88–1.28							
NLCS (Hogervorst <i>et al.</i> , 2007)	1350	0.93	0.73–1.19	0.99	0.92–1.06	767	1.10	0.80–1.52	1.01	0.93–1.11
Danish Diet, Cancer and Health ^b (Olesen <i>et al.</i> , 2008)	374			1.05	0.66–1.69	249 ^c			1.5	0.6–3.6
SMC (Larsson <i>et al.</i> , 2009b)	2952	0.91	0.80–1.02			NR	0.91	0.65–1.27		
NHS II (Wilson <i>et al.</i> , 2009b)	1179	0.92	0.76–1.11			738	0.82	0.64–1.05		
NLCS (Pedersen <i>et al.</i> , 2009)	1690	0.92	0.73–1.15	0.97	0.91–1.03	953	1.15	0.86–1.53	1.01	0.93–1.10
NHS I (Wilson <i>et al.</i> , 2010)	6301	0.95	0.87–1.03			2752	0.89	0.78–1.02		
UKWCS (Burley <i>et al.</i> , 2010)	1084	1.16	0.88–1.52	1.08	0.98–1.18	607	0.98	0.69–1.40	1.06	0.93–1.21
Endometrial cancer										
NLCS (Hogervorst <i>et al.</i> , 2007)	221	1.29	0.81–2.07	1.04	0.91–1.19	150	1.99	1.12–3.52	1.12	0.95–1.33
SMC (Larsson <i>et al.</i> , 2009e)	687	0.96	0.76–1.21			169	1.20	0.76–1.90		
NHS I (Wilson <i>et al.</i> , 2010)	484	1.41	1.01–1.97			257	1.43	0.90–2.28		
Ovarian cancer										
Italy ^a (Pelucchi <i>et al.</i> , 2006)	1031	0.97	0.73–1.31							
NLCS (Hogervorst <i>et al.</i> , 2007)	195	1.78	1.10–2.88	1.11	0.99–1.25	129	2.22	1.20–4.08	1.17	1.01–1.36
SMC (Larsson <i>et al.</i> , 2009c)	368	0.86	0.63–1.16			75	0.97	0.49–1.93		
NHS I (Wilson <i>et al.</i> , 2010)	416	1.25	0.88–1.77			156	1.19	0.66–2.15		
Prostate cancer										
Italy ^a (Pelucchi <i>et al.</i> , 2006)	1294	0.92	0.69–1.23							
NLCS (Hogervorst <i>et al.</i> , 2008a)	2246	1.06	0.87–1.30	1.01	0.96–1.07	347	0.72	0.43–1.20	0.95	0.83–1.09
CAPS ^a (Wilson <i>et al.</i> , 2009a)	1438	0.97	0.75–1.27	0.99	0.92–1.06	NR ^d			0.97	0.86–1.08
Cohort of Swedish Men (Larsson <i>et al.</i> , 2009d)	2696	0.88	0.70–1.09			1088	0.91	0.74–1.13		
ATBC (Hirvonen <i>et al.</i> , 2010)	799	1.05	0.83–1.32							
HPFS (Wilson <i>et al.</i> , 2011)	5025	1.02	0.92–1.13			1925	1.01	0.85–1.19		
Lung cancer										
NLCS (Hogervorst <i>et al.</i> , 2009a)										
Men	1600	1.03	0.77–1.39	1.03	0.96–1.11	61	2.18	0.61–7.82	0.93	0.66–1.32
Women	295	0.45	0.27–0.76	0.82	0.69–0.96	73	0.62	0.33–1.16	0.78	0.61–1.00
ATBC (Hirvonen <i>et al.</i> , 2010)	1703	1.18	1.01–1.38							
Pancreatic cancer										
NLCS (Hogervorst <i>et al.</i> , 2008b)	349	0.98	0.68–1.40	1.06	0.96–1.17	166 ^c	0.80	0.48–1.32	1.07	0.93–1.24
ATBC (Hirvonen <i>et al.</i> , 2010)	192	1.00	0.62–1.62							
Italy ^a (Pelucchi <i>et al.</i> , 2011a)	326	1.49	0.83–2.70	1.01	0.92–1.10	NR			1.01	0.86–1.18
Renal cell cancer										
Sweden ^a (Mucci <i>et al.</i> , 2003b)	133	0.8	0.4–1.7			99 ^c	0.7	0.3–1.7		
Sweden ^a (Mucci <i>et al.</i> , 2004)	379	1.1	0.7–1.8			NR ^c	1.0			
Italy ^a (Pelucchi <i>et al.</i> , 2007)	767	1.20	0.88–1.63	1.05 ^e	0.94–1.16					
NLCS (Hogervorst <i>et al.</i> , 2008a)	339	1.59	1.09–2.30	1.10	1.01–1.21	93	1.51	0.73–3.10	1.09	0.89–1.34
ATBC (Hirvonen <i>et al.</i> , 2010)	184	1.28	0.76–2.15							
Bladder cancer										
Sweden ^a (Mucci <i>et al.</i> , 2003b)	263	0.8	0.5–1.5			135 ^c	0.7	0.3–1.6		
NLCS (Hogervorst <i>et al.</i> , 2008a)	1210	0.91	0.73–1.15	1.00	0.95–1.06	155	0.55	0.33–0.93	0.90	0.76–1.05
Colorectal cancer										
Sweden ^a (Mucci <i>et al.</i> , 2003b)	591	0.6	0.4–1.0			457 ^c	0.6	0.3–1.0		
Italy and Switzerland ^a (Pelucchi <i>et al.</i> , 2006)	2280	0.97	0.80–1.18							
SMC (Mucci <i>et al.</i> , 2006)	741	0.9	0.7–1.3							
NLCS (Hogervorst <i>et al.</i> , 2008b)	2190	1.00	0.84–1.20	1.00	0.96–1.06	717	1.19	0.88–1.63	1.03	0.94–1.12
Cohort of Swedish Men (Larsson <i>et al.</i> , 2009a)	676	0.95	0.74–1.20			NR	0.97	0.64–1.50		
ATBC (Hirvonen <i>et al.</i> , 2010)	316	0.93	0.65–1.34							
Oral cavity/pharyngeal cancer										
Italy and Switzerland ^a (Pelucchi <i>et al.</i> , 2006)	749	1.12	0.76–1.66							
NLCS (Oral cavity) (Schouten <i>et al.</i> , 2009)	101	0.72	0.36–1.42	0.90	0.73–1.10	39 ^c			1.06	0.84–1.33
NLCS (Pharyngeal) (Schouten <i>et al.</i> , 2009)	83	0.61	0.33–1.12	0.74	0.53–1.03					
Laryngeal cancer										
Italy and Switzerland ^a (Pelucchi <i>et al.</i> , 2006)	527	1.23	0.80–1.90							
NLCS (Schouten <i>et al.</i> , 2009)	180	0.93	0.54–1.58	1.05	0.91–1.21	34 ^c			0.82	0.53–1.29
Esophageal cancer										
Italy and Switzerland ^a (Pelucchi <i>et al.</i> , 2006)	395	1.10	0.65–1.86							
NLCS (Hogervorst <i>et al.</i> , 2008b)	216	0.83	0.54–1.30	0.96	0.85–1.09	83 ^c	0.73	0.36–1.47	0.92	0.76–1.11
Sweden ^a (Lin <i>et al.</i> , 2011)	594	1.23	1.02–1.75			363 ^c	1.46	0.96–2.21		
Stomach cancer										
NLCS (Hogervorst <i>et al.</i> , 2008b)	563	1.06	0.78–1.45	1.02	0.94–1.10	250 ^c	1.43	0.92–2.24	1.09	0.98–1.22
ATBC (Hirvonen <i>et al.</i> , 2010)	224	0.96	0.60–1.53							

Table 1 (continued)

Study	Total population					Never smokers				
	Cases	High vs. low estimated intake		Continuous (per 10 µg/day)		Cases	High vs. low estimated intake		Continuous (per 10 µg/day)	
		RR	95% CI	RR	95% CI		RR	95% CI	RR	95% CI
Brain cancer										
NLCS (Hogervorst <i>et al.</i> , 2009b)	216	0.87	0.54–1.41	1.02	0.89–1.16	69	0.87	0.46–1.63	1.07	0.83–1.39

ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CAPS, Cancer of the Prostate in Sweden; CI, confidence interval; HPFS, Health Professionals' Follow-Up Study; NHS, Nurses' Health Study; NLCS, Netherlands Cohort Study; NR, Not Reported; RR, relative risk; SMC, Swedish Mammography Cohort; UKWCS, UK Women's Cohort Study.

^aCase-control study; reported risk estimates are odds ratios.

^bNested biomarker case-control study; risk estimates are incidence rate ratios for a 10-fold increase in the biomarker (acrylamide-hemoglobin adduct) concentration rather than exposure estimated from a food frequency questionnaire.

^cNonsmokers (includes former smokers).

^dThirty-nine percent of all cases were reported to be never smokers.

^eThe continuous measurement unit was 1 SD of the distribution in controls (18.1 µg/day).

No association was reported between dietary acrylamide and breast cancer in the NLCS (Hogervorst *et al.*, 2007). A total of 1350 cases of breast cancer were observed during 11.3 years of follow-up, with an RR of 0.93 for the highest quintile of acrylamide intake; the corresponding RR was 1.10 among never smokers. A later and larger report from the NLCS (Pedersen *et al.*, 2009) also found no association between acrylamide and postmenopausal breast cancer overall, but did report a moderately increased RR of 1.43 (95% CI 0.83–2.46) for hormone receptor-positive breast cancer in a subgroup of never smoking postmenopausal women with the highest quintile of acrylamide intake. However, neither the RR nor the dose-response relation was statistically significant, and this RR was one of over 100 subgroup RRs reported in the analysis. Hence, the role of chance cannot be excluded with any confidence.

Consistent with the results of cohort studies, a hospital-based case-control study of multiple cancers in Italy and Switzerland (Pelucchi *et al.*, 2006), including 2900 breast cancer cases, reported an odds ratio (OR) of 1.06 for the highest quintile of acrylamide intake. Dietary data were obtained using a 78-item FFQ, with acrylamide content derived from WHO estimates. In this study, smoking habits were assessed during a structured in-person interview and were considered as a potential confounder in the analyses of all cancers, with the exception of breast and ovarian.

Olesen *et al.* (2008) carried out a biomarker case-control study, nested in the Danish Diet, Cancer and Health Study, of acrylamide-hemoglobin adducts in relation to postmenopausal breast cancer. Adduct levels were similar among cases and controls, and were about three times higher among smokers compared with nonsmoking women. Among nonsmokers, for whom adduct levels are assumed to largely represent acrylamide exposure from the diet, there was no statistically significant association

between adduct levels and the risk of breast cancer. On using multiple models to adjust for various combinations of smoking and nonsmoking confounding variables, a statistically significantly elevated RR was found in one statistical model for the subgroup of ER + breast cancers associated with the acrylamide-hemoglobin adduct level among smokers (RR = 4.9; 95% CI 1.2–20). Associations of adduct levels with cancer among smokers should not be interpreted as evidence of carcinogenicity of dietary acrylamide intake; the RR among nonsmokers in this analysis was not significantly elevated (RR = 1.9; 95% CI 0.7–5.6). The association between dietary acrylamide intake and the subgroup of ER + breast cancers has not been confirmed in other studies that have examined this issue (Larsson *et al.*, 2009b; Wilson *et al.*, 2009b, 2010).

Pelucchi *et al.* (2011b) published a meta-analysis of epidemiologic studies evaluating dietary acrylamide and cancer. On the basis of the five breast cancer studies published at that time, the pooled RR for the highest intake was 0.97 (95% CI 0.89–1.06). Results published since the meta-analysis have been consistent with the earlier findings (Burley *et al.*, 2010; Wilson *et al.*, 2010). Thus, the collective evidence from numerous large cohort studies suggests that the intake of foods high in acrylamide is not a risk factor for breast cancer.

Endometrial cancer

The risk of endometrial cancer in relation to dietary acrylamide intake has been evaluated in the three large prospective studies, the NLCS, SMC, and NHS I (Hogervorst *et al.*, 2007; Larsson *et al.*, 2009e; Wilson *et al.*, 2010). In the NLCS, there was no significant association overall between increasing levels of acrylamide intake and endometrial cancer, with an RR of 1.29 (95% CI 0.81–2.07) for the highest quintile (Hogervorst *et al.*, 2007). In subgroup analyses by smoking status, a significantly elevated RR was observed only in the

highest quintile of acrylamide intake among women who never smoked (RR = 1.99; 95% CI 1.12–3.52), on the basis of 40 observed cases of endometrial cancer. An increased risk for endometrial cancer among those who consumed the highest levels of acrylamide was also observed among women in the NHS I (RR = 1.41; 95% CI 1.01–1.97) (Wilson *et al.*, 2010); however, in extensive subgroup analyses, the higher risk among never smokers observed in the Dutch study was not confirmed in the NHS I population, but rather the increased risk for endometrial cancer was found only among those with a BMI less than 25. In the SMC (Larsson *et al.*, 2009e), there was no association between acrylamide intake and endometrial cancer after more than 17 years of follow-up, with an RR of 0.96 for the highest quartile of acrylamide intake. When the cohort was restricted to those for whom data were available on smoking, the RRs among those with the highest acrylamide intake were 1.12 (95% CI 0.79–1.59) adjusted for smoking and 1.20 (95% CI 0.76–1.90) in the subgroup of never smokers.

In the recent meta-analysis, which did not include the NHS I results, the summary RR for endometrial cancer for the highest level of acrylamide intake was 1.03 (95% CI 0.80–1.33), whereas the corresponding pooled RR on the basis of available data for never smokers was 1.50 (95% CI 0.92–2.45), largely explained by the Dutch study (Pelucchi *et al.*, 2011b). The increased RR among never smokers observed in the Dutch cohort study was not found in the NHS I population or the SMC cohort. Hence, there is little consistency across three large cohort studies that high intake of acrylamide is associated with an increased risk of endometrial cancer overall or among never smokers.

Despite an overall pooled RR close to unity, Pelucchi *et al.* (2011b) suggested that additional research is needed before a final conclusion can be reached on an association between dietary acrylamide intake and endometrial cancer. It is noteworthy, however, that there is no increased risk of endometrial cancer among cigarette smokers, who have substantially higher acrylamide levels; in fact, the association between cigarette smoking and endometrial cancer appears to be inverse (Baron, 1996; Vineis *et al.*, 2004), suggesting that any positive association between the comparatively small contribution of dietary acrylamide and endometrial cancer is unlikely. Moreover, coffee, similar to smoking, has been shown to lower the risk of endometrial cancer (Bravi *et al.*, 2009; Arab 2010; Yu *et al.*, 2011); this inverse association is consistent and evident in many populations, including in the NHS I population (Je *et al.*, 2011), in which coffee is the major contributor to dietary acrylamide intake (Wilson *et al.*, 2010). Thus, the absence of an association between dietary acrylamide intake and endometrial cancer is consistent with the absence of an increased risk of endometrial cancer among those who smoke or

consume large amounts of coffee, which together account for the largest source of acrylamide exposure overall.

Ovarian cancer

The same three cohort studies, as well as a case–control study in Italy (Pelucchi *et al.*, 2006), have evaluated ovarian cancer in relation to acrylamide intake, with similarly inconsistent results. In the NLCS (Hogervorst *et al.*, 2007), the RR for ovarian cancer was significantly increased among those in the highest category of daily acrylamide intake (RR = 1.78; 95% CI 1.10–2.88) and among the subgroup of never smokers (RR = 2.22; 95% CI 1.20–4.08); as was the pattern for endometrial cancer in this cohort, RRs for virtually all other quintiles of acrylamide were not significantly elevated. The NHS I did not confirm the results of the Dutch study, as there was no significantly increased risk for ovarian cancer associated with acrylamide intake either overall (RR = 1.25; 95% CI 0.88–1.77) or restricted to never smokers (RR = 1.19; 95% CI 0.66–2.15) (Wilson *et al.*, 2010). Further, the RRs in the SMC study for ovarian cancer associated with the highest levels of acrylamide intake were below 1.0, both overall and among never smokers (Larsson *et al.*, 2009c), as well as in the Italian case–control study of 1031 ovarian cancer cases (Pelucchi *et al.*, 2006). Pelucchi *et al.* (2011b) reported a pooled RR for ovarian cancer close to unity (RR = 1.09; 95% CI 0.76–1.57), but suggested a need for additional research. Again, as with endometrial cancer, the lack of an association of ovarian cancer with dietary acrylamide intake is consistent with the absence of an increased risk of ovarian cancer among cigarette smokers (Vineis *et al.*, 2004), who have much higher acrylamide levels.

Prostate cancer

A conjectured hormonal action of acrylamide has led to interest in prostate cancer in the investigation of dietary acrylamide carcinogenicity (Hogervorst *et al.*, 2008a; Wilson *et al.*, 2011). To date, the association between acrylamide intake and prostate cancer has been examined in two case–control studies and four large, prospective cohorts (Pelucchi *et al.*, 2006; Hogervorst *et al.*, 2008a; Larsson *et al.*, 2009d; Wilson *et al.*, 2009a, 2011; Hirvonen *et al.*, 2010). Acrylamide intake was generally similar across studies, with an average of about 20 µg/day.

The Italian case–control study analyzed 1294 cases of prostate cancer and 1451 controls (Pelucchi *et al.*, 2006). The OR for the highest (36.4 µg/day) versus the lowest (12.4 µg/day) quintile was 0.92 (95% CI 0.69–1.23), adjusted for smoking habits. The second case–control study, Cancer of the Prostate in Sweden, included 1438 cases and 1066 controls (Wilson *et al.*, 2009a). This study was unique in that it not only evaluated FFQ-estimated acrylamide intake but also measured acrylamide-hemoglobin adduct concentration in a percentage of the cases and controls (11 and 15%, respectively), and found

a low to moderate correlation between the two measures ($r = 0.15$ for cases and $r = 0.35$ for controls). As in the Italian study, no trend for an increasing risk of prostate cancer was observed with increasing quintile of acrylamide intake; the OR for Q5 (56–125 $\mu\text{g/day}$) versus Q1 (8–33 $\mu\text{g/day}$) was 0.97 (95% CI 0.75–1.27). The results were similar for the smaller analysis of 170 cases and 161 controls with available acrylamide-hemoglobin adduct concentration; none of the ORs for high dietary intake of acrylamide was significantly increased, irrespective of adduct concentration quartile or cancer severity (e.g. localized, advanced).

The first prospective study to examine estimated dietary acrylamide intake and prostate cancer was the NLCS (Hogervorst *et al.*, 2008a). After 13.3 years of follow-up, 2246 prostate cancer cases were identified, with virtually no difference in the estimated mean dietary acrylamide intake between cases (22.4 $\mu\text{g/day}$) and the subcohort (22.6 $\mu\text{g/day}$). All RRs were close to 1.0, with an RR for the highest quintile of acrylamide intake of 1.06 (95% CI 0.87–1.30). When the analysis was restricted to never smokers, an RR of 0.72 (95% CI 0.43–1.20) for prostate cancer was observed for the highest quintile and the inverse association was more pronounced in the subgroup of advanced prostate cancer cases ($N = 117$), with an RR of 0.57 (95% CI 0.27–1.17) for the highest quintile of intake (P trend = 0.10).

Larsson *et al.* (2009d) analyzed 2696 prostate cancer cases in 45 306 men within the Cohort of Swedish Men, with similar results. The RR for the highest (≥ 43.4 $\mu\text{g/day}$) versus the lowest (< 28.3 $\mu\text{g/day}$) quintile was 0.88 (95% CI 0.70–1.09) for total prostate cancer, and the results were similarly close to 1.0 for localized and advanced prostate cancers. The decrease in risk was more apparent for never smokers with advanced prostate cancer ($N = 351$), with an RR of 0.75 (95% CI 0.51–1.10) for the highest consumption quintile (P trend = 0.15). In 2010, the association between prostate cancer and dietary acrylamide intake was examined among 799 cases in the Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) cohort of 27 111 male smokers. RRs were close to unity, with an RR of 1.05 (95% CI 0.83–1.32) for the highest versus the lowest intake levels (55.7 vs. 21.9 $\mu\text{g/day}$) (Hirvonen *et al.*, 2010).

The first US study to investigate dietary acrylamide and prostate cancer was the Health Professionals' Follow-up Study in 2011 (Wilson *et al.*, 2011). Using FFQ data updated every 4 years, the analysis of 5025 prostate cancers (642 lethal) within a cohort of 47 896 revealed somewhat similar patterns. RRs for all cancers, high grade, low grade, and localized cases were typically above 1.0, and decreased with increasing quintiles of estimated acrylamide intake. The RR for all prostate cancer was 1.02 (95% CI 0.92–1.13) for the highest intake level. RRs for lethal or advanced cancer were close to or below 1.0.

In summary, six studies have examined the relation between dietary acrylamide and prostate cancer. There is no evidence of a positive association for prostate cancer overall, or by disease severity (e.g. localized, advanced, lethal), or when using the concentration of acrylamide-hemoglobin adducts as a quantitative marker for acrylamide exposure, as opposed to an FFQ-estimated exposure. In the recent meta-analysis (Pelucchi *et al.*, 2011b), which included four of the five European studies (the ATBC study not included), the pooled RR for the highest versus the lowest category of acrylamide intake was 0.96 (95% CI 0.86–1.09); for a 10 $\mu\text{g/day}$ increase in acrylamide intake, the pooled continuous RR was 0.99 (95% CI 0.96–1.01).

Lung cancer

Not only is smoking a powerful confounder in dietary epidemiologic studies of lung cancer, tobacco smoke is also a major source of acrylamide exposure. The two studies of lung cancer and dietary acrylamide intake to date, the NLCS (Hogervorst *et al.*, 2009a) and ATBC (Hirvonen *et al.*, 2010), both attempted to control for this confounding by adjusting for the number of cigarettes per day and the duration of smoking.

In the NLCS, male subcohort controls consumed 22.6 $\mu\text{g/day}$, whereas women consumed 21.0 $\mu\text{g/day}$; male cases typically consumed 22.7 $\mu\text{g/day}$, depending on histological type, and female cases typically consumed 20.7 $\mu\text{g/day}$, depending on cell histology (Hogervorst *et al.*, 2009a). No significant association was observed in the NLCS after 13.3 years of follow-up between lung cancer and dietary acrylamide for men ($N = 1600$) and an inverse association was observed for women ($N = 295$). The continuous RR per 10 $\mu\text{g/day}$ increase for men was 1.03, and the RR of lung cancer for the highest intake level was similarly 1.03. The RRs were similarly close to 1.0 for all histological subtypes. Among the small number of never smokers with lung cancer ($N = 61$), RRs were nonsignificantly elevated by tertiles of acrylamide intake. For women, the continuous RR was 0.82 (95% CI 0.69–0.96), and a statistically significant decreasing trend (P trend = 0.01) was observed with increasing quintiles, with an RR = 0.45 (95% CI 0.27–0.76) for the top quintile. A similar inverse trend for acrylamide consumption was observed for never-smoking women and for all histological subtypes, but was statistically significant only for adenocarcinomas (AC; Hogervorst *et al.*, 2009a).

In the ATBC cohort with 1703 lung cancers among male smokers after 10.2 years of follow-up, estimated acrylamide intake was higher, with a median 36.8 $\mu\text{g/day}$ overall, and the adjusted RR for the highest quintile of dietary acrylamide was 1.18 (95% CI 1.01–1.38), with no significant dose trend (P trend = 0.11) (Hirvonen *et al.*, 2010). Both the estimated acrylamide intake and the lung cancer RR were higher in the ATBC than among men in the NLCS, but the likely potential for residual

confounding by smoking cannot be ruled out in the ATBC, a study consisting exclusively of male smokers. Overall, the evidence suggests that acrylamide does not increase the risk for lung cancer. Only the NLCS has studied this relationship in women, finding a statistically significant inverse association with increasing acrylamide exposure.

Pancreatic cancer

Since the report of a nonsignificant increase in pancreatic cancer risk with acrylamide exposure (SMR = 2.22; 95% CI 0.72–5.19) in an occupational mortality study of acrylamide workers (Swaen *et al.*, 2007), three epidemiologic studies have evaluated the relation between dietary acrylamide and pancreatic cancer. The NLCS examined pancreatic cancer among 349 cases, after 13.3 years of follow-up (Hogervorst *et al.*, 2008b). No statistically significantly elevated RRs were observed, with an RR of 0.98 for the highest quintile. RRs restricted to microscopically verified cancers ($N=233$) were similar, whereas those for nonsmokers ($N=166$) were somewhat lower (Q5 RR = 0.80; 95% CI 0.48–1.32). A suggestion of effect modification by obesity in the analysis of microscopically verified cancers is likely due to chance arising from multiple subgroup comparisons. The ATBC Study in Finland was the only other prospective study to evaluate pancreatic cancer and dietary acrylamide, with 192 cases after 10.2 years of follow-up (Hirvonen *et al.*, 2010). No significant increase in risk was observed, and no trend was apparent across quintiles (P trend = 0.89); the RR for the highest quintile was 1.0.

In 2011, an Italian hospital-based case-control study also reported no statistically significant association between acrylamide intake and pancreatic cancer (Pelucchi *et al.*, 2011a). The estimated mean daily acrylamide intake was 33.5 μg among 326 pancreatic cancer cases and 32.2 μg among 652 controls. ORs were increased for quintiles 2–5, with an OR for the highest quintile ($>44.7 \mu\text{g/day}$) compared with the lowest ($<16.2 \mu\text{g/day}$) of 1.49 (95% CI 0.83–2.70); no dose-response trend was observed (P trend = 0.21). The continuous (per 10 $\mu\text{g/day}$) OR was 1.01 (95% CI 0.92–1.10), and was similar among smokers and never smokers. Pooling data from the Dutch study, Pelucchi *et al.* (2011a) compared each intake quintile with the first and observed nonsignificantly increased RRs, ranging between 1.13 (95% CI 0.77–1.67) and 1.18 (95% CI 0.61–2.26); the continuous pooled RR (per 10 $\mu\text{g/day}$ increase) was 1.03 (95% CI 0.97–1.10). Overall, pancreatic cancer shows no relation with dietary intake of acrylamide.

Renal cell cancer

One of the earliest epidemiologic studies of dietary acrylamide intake, a population-based case-control study in Sweden, evaluated 133 cases of renal cell cancer (Mucci *et al.*, 2003b), and since then, renal cell cancer has

been examined in numerous studies. Mucci *et al.* (2003b) reported an OR of 0.8 (95% CI 0.4–1.7) for the highest quartile of acrylamide intake; the corresponding ORs were 0.7 (95% CI 0.3–1.7) and 1.3 (95% CI 0.2–6.3) among nonsmokers and current smokers, respectively. The inclusion of additional data on acrylamide consumed through coffee intake, which were not available at the time of the original publication (Mucci *et al.*, 2003a), expanded the range of acrylamide exposure in this population and provided a more complete estimate of acrylamide intake; when data on coffee consumption were included, the ORs for kidney cancer associated with higher quartiles of acrylamide intake decreased slightly.

A second case-control study in Sweden (Mucci *et al.*, 2004), including 379 renal cell cancer cases and 353 population controls, reported ORs of approximately 1.0 for those in the highest quartile ($>31.9 \mu\text{g/day}$) of acrylamide intake compared with the lowest ($<20.1 \mu\text{g/day}$), irrespective of smoking status. Similarly, no association was apparent between acrylamide intake and renal cell cancer in an Italian case-control study (Pelucchi *et al.*, 2007), which included 767 cases and 1534 controls, with an OR of 1.20 (95% CI 0.88–1.63) for the highest level of intake, adjusted for smoking status. In the ATBC cohort study of Finnish male smokers, 184 cases of renal cell cancer were observed during an average of 10.2 years of follow-up; the RR for renal cell cancer among those with the highest acrylamide intake was 1.28 (95% CI 0.76–2.15), on the basis of 38 cases (Hirvonen *et al.*, 2010). In the NLCS analysis of 339 cases of renal cell cancer (Hogervorst *et al.*, 2008a), RRs of 1.25 (95% CI 0.86–1.83), 1.48 (95% CI 1.02–2.15), 1.23 (95% CI 0.83–1.81), and 1.59 (95% CI 1.09–2.30) were reported for consumption levels of acrylamide in quintiles 2–5. Although the P trend was significant ($P=0.04$), the RRs do not indicate a monotonic increase in risk with increasing acrylamide intake. Among never smokers, there was even less evidence of a dose effect (P trend = 0.68).

Pelucchi *et al.* (2011b), in their meta-analysis reported a pooled nonsignificant RR of 1.12 (95% CI 0.80–1.57) for the highest versus the lowest level of acrylamide intake and renal cell cancer. They concluded, partly on the basis of the significantly increased kidney cancer risk reported for the highest level of dietary acrylamide consumption in the NLCS, that an association between dietary acrylamide exposure and kidney cancer ‘cannot be excluded’ (Pelucchi *et al.*, 2011b). After an extensive review of the results of all studies examining renal cell cancer, we conclude that there is no consistent evidence of an association between dietary acrylamide intake and renal cell cancer.

Bladder cancer

Bladder cancer in relation to dietary acrylamide intake has been evaluated in two studies. In the prospective NLCS

(Hogervorst *et al.*, 2008a), which included 1210 bladder cancer cases, almost all RRs were below 1.0, and often significantly so, among never smokers. For the highest quintile of acrylamide intake compared with the lowest, the RR for bladder cancer was 0.91 (95% CI 0.73–1.15) overall and 0.55 (95% CI 0.33–0.93) among never smokers. A Swedish population-based case–control study (Mucci *et al.*, 2003b) of 263 bladder cancer cases and 538 controls reported no association between acrylamide intake and bladder cancer either overall (OR for highest vs. lowest quartile = 0.8; 95% CI 0.5–1.5) or among nonsmokers (OR = 0.7; 95% CI 0.3–1.6) or current smokers (OR = 1.0; 95% CI 0.4–2.3). After the inclusion of data on coffee intake, ORs for bladder cancer remained close to 1.0 for all levels of acrylamide intake (Mucci *et al.*, 2003a).

Colorectal cancer

Six European studies, including two case–control (Mucci *et al.*, 2003b; Pelucchi *et al.*, 2006) and four cohort studies (Mucci *et al.*, 2006; Hogervorst *et al.*, 2008b; Larsson *et al.*, 2009a; Hirvonen *et al.*, 2010), have analyzed the relation between colorectal cancer and dietary acrylamide. With the exception of one statistically significant inverse trend in risk of colorectal cancer among nonsmokers in Sweden (Mucci *et al.*, 2003b), risk estimates have been consistently close to 1.0 both for categories of total dietary exposure and for continuous 10 µg/day increases in dietary acrylamide.

In 2003, Mucci *et al.* reported a strong reduction in risk and the only significant trend of the six studies of colorectal cancer and dietary acrylamide intake. For 591 large bowel cancers in Sweden, the adjusted OR was 0.6 (95% CI 0.4–1.0, *P* trend 0.01) for the highest versus the lowest quartile of acrylamide intake; the statistically significant inverse trend was limited to the subgroup of nonsmokers, although the inverse relationship was still present among current smokers (Q4 OR = 0.7; 95% CI 0.3–2.1) (Mucci *et al.*, 2003b). No significant trends or deviations in risk were observed with total colorectal cancer, colon cancer, or rectal cancer and estimated dietary acrylamide intake in the Italian and Swiss case–control study with 2280 total colorectal cancer cases (Pelucchi *et al.*, 2006), the SMC with 741 cases (Mucci *et al.*, 2006), the NLCS with 2190 cases (Hogervorst *et al.*, 2008b), the Cohort of Swedish Men with 676 cases (Larsson *et al.*, 2009a), or the ATBC with 316 cases (Hirvonen *et al.*, 2010).

In the meta-analysis by Pelucchi *et al.* (2011b), combining data on 5887 colorectal cancer cases from five of the six studies examining colorectal cancer (excluding the ATBC cohort), the pooled RR for colorectal cancer was 0.93 (95% CI 0.82–1.05) for the highest quartile of acrylamide exposure versus the lowest. For a 10 µg/day increase in the estimated dietary acrylamide intake, the pooled RR

was 0.99 (95% CI 0.95–1.02). The results were similar for the 3813 colon cancer cases, with RRs of 1.01 (95% CI 0.89–1.15) for the quartile analysis and 1.01 (95% CI 0.99–1.04) for the continuous, and for 1899 rectal cancer cases, with RRs of 0.95 (95% CI 0.79–1.13) and 1.00 (95% CI 0.97–1.03), respectively (Pelucchi *et al.*, 2011b).

Despite a few, sporadic inverse associations found to date, the collective evidence suggests that dietary acrylamide intake is not associated with a risk of colorectal cancer.

Oral cavity/pharyngeal and laryngeal cancers

Two studies, one case–control and one prospective cohort study, have considered dietary acrylamide in relation to cancers of the oral cavity and the pharynx or larynx. In 2006, Pelucchi *et al.* (2006) analyzed 749 cases of oral and pharynx cancer and 527 cases of laryngeal cancer as part of the hospital-based case–control study in Italy and Switzerland. For cancer of the oral pharynx, ORs for all quintiles were above 1.0, but not significantly so, and the OR for the highest quintile (> 40.4 µg/day) versus the lowest (< 12.9 µg/day) was 1.12 (95% CI 0.76–1.66), adjusted for smoking and alcohol intake. The corresponding OR for laryngeal cancer among those in the highest quintile of acrylamide intake was 1.23 (95% CI 0.80–1.90). Schouten *et al.* (2009) reported similar results in the NLCS for 101 oral cavity cancers, 83 oropharyngeal and hypopharyngeal cancers, 180 laryngeal cancers, and 357 total head and neck cancers (which included cancer of the thyroid). The RRs for the highest quintiles were 0.72 (95% CI 0.36–1.42) for oral cavity cancer; 0.61 (95% CI 0.33–1.12) for oropharyngeal and hypopharyngeal cancer; 0.93 (95% CI 0.54–1.58) for laryngeal cancer; and 0.74 (95% CI 0.50–1.09) for all head and neck cancers. The results were generally similar for men and women and for smokers and nonsmokers. Overall, the available evidence does not support an association between dietary acrylamide and cancers of the oral cavity and the pharynx or larynx.

Esophageal cancer

To date, two case–control and one cohort study have evaluated dietary acrylamide and the risk of esophageal cancer. Three hundred and ninety-five esophageal cancers were observed in the 2006 Italian and Swiss case–control study (Pelucchi *et al.*, 2006). All quintile ORs were close to 1.0. The OR for the highest (> 39.6 µg/day) versus the lowest intake quintile (< 13.2 µg/day) was 1.10 (95% CI 0.65–1.86), adjusted for smoking status. The NLCS studied 115 AC and 90 squamous cell carcinomas (SCC) of the esophagus. The continuous RR (per 10 µg/day increase) was 0.96 overall (95% CI 0.85–1.09), 1.00 (95% CI 0.85–1.17) for AC, and 0.95 (95% CI 0.78–1.16) for SCC. The results were similar for nonsmokers (83 cases), with a continuous RR of 0.92 (95% CI 0.76–1.11) (Hogervorst *et al.*, 2008b). The 2011 meta-analysis of these two studies, with a total of 611

esophageal cancer cases, reached similar conclusions. The RR for the highest intake level was 0.93 (95% CI 0.66–1.31) and the continuous RR was 0.98 (95% CI 0.91–1.06) (Pelucchi *et al.*, 2011b).

A recent population-based case–control study in Sweden reported similarly null results for esophageal AC but different results for SCC and total esophageal cancer (Lin *et al.*, 2011). In analyses on the basis of quartiles of acrylamide intake (in contrast to quintiles used in most other studies of dietary acrylamide), a statistically significant increasing trend (P trend = 0.02) was observed for SCC, with ORs increasing monotonically from 1.30 (95% CI 0.74–2.31) to 1.56 (95% CI 0.86–2.85) for the top quartile of intake. Associations with SCC were stronger when restricted to overweight and obese participants and nonsmokers.

In contrast to the NLCS and the Italian and Swiss case–control study, both of which collected dietary intake information for 1 and 2 years (respectively) before interview, the dietary assessment for the Swedish case–control study inquired about intake 20 years before the diagnosis of cancer (Pelucchi *et al.*, 2006; Hogervorst *et al.*, 2008b; Lin *et al.*, 2011). Thus, one explanation for the disparate findings regarding SCC esophageal cancer may be information bias resulting from differential recall on the part of cases and controls of their diets 20 years before the interview. Although it may be that diet decades ago is more relevant to the risk of cancer in case–control studies than more recent exposures (i.e. a latency period between exposure and diagnosis), the NLCS cohort investigation evaluated cancers over a relatively long follow-up period (average 13.3 years) and observed no association between acrylamide intake in the year before enrollment and the risk of esophageal cancer. In addition, residual confounding due to smoking or alcohol drinking alone or combined, which are the predominant causes of SCC of the esophagus, cannot be ruled out in the examination of potentially small effects of dietary acrylamide intake on the risk of SCC. Taken as a whole, the collective evidence does not support an association between esophageal cancer and dietary acrylamide.

Stomach cancer

Results are available from two studies, both prospective cohorts, on stomach cancer and dietary acrylamide. In the NLCS, the continuous (per 10 µg/day) RR was 1.02 (95% CI 0.94–1.10) for 563 cases. No trend was observed over quintiles of intake, with an RR of 1.06 (95% CI 0.78–1.45) for the highest quintile. Similar results were observed for the gastric cardia (N = 143) and distal gastric cancer (N = 238) subtypes. Among nonsmokers (N = 250 total, N = 66 for gastric cardia, N = 104 for distal cancer), RRs were typically greater than 1.0, although no confidence intervals excluded 1.0 and the P values for trend remained greater than 0.15 (Hogervorst *et al.*,

2008b). In the ATBC cohort, 224 total gastric cancers were observed. RRs with increasing quintiles of acrylamide intake showed no clear trend, with an RR of 0.96 (95% CI 0.60–1.53) for the highest level (Hirvonen *et al.*, 2010). The results from these two prospective cohort studies do not support an association between dietary acrylamide and stomach cancer.

Brain cancer

The NLCS has been the only study to specifically evaluate dietary acrylamide and adult brain cancer. Hogervorst *et al.* (2009b) analyzed total brain cancer (N = 216), microscopically verified brain cancer (N = 170), astrocytic gliomas (N = 151), and high-grade astrocytic gliomas (N = 132) within the NLCS. Typical acrylamide intake was 22.1 µg/day for brain cancer cases and 21.8 µg/day for the subcohort. No association was found for a continuous 10 µg/day increase in acrylamide intake (total brain cancer RR = 1.02; 95% CI 0.89–1.16) or for the highest intake quintile (total brain cancer RR = 0.87; 95% CI 0.54–1.41). The results were similar for each histological type and for the smaller group of never smokers (N = 69 total brain cancers), for whom the RR for continuous acrylamide intake was 1.07 and the RR for the highest tertile of intake was 0.87 (95% CI 0.46–1.63). In addition, brain cancer was not associated with either Dutch spiced cake or coffee consumption, which contributed the most dietary acrylamide in the NLCS cohort (Hogervorst *et al.*, 2009b).

Discussion

In fewer than 10 years since the initial report indicating the formation of acrylamide during cooking of common foods, and in 8 years since the publication of the first epidemiologic study of dietary acrylamide in relation to cancer (Mucci *et al.*, 2003b), almost 30 papers have been published from epidemiologic studies examining dietary acrylamide in relation to cancer. These studies, in multiple populations, using different study designs, have evaluated the potential risk of cancer for multiple organs and with multiple studies per target organ. Analyses have been carried out overall and also in subgroups defined by smoking, obesity, histologic subtype, and tumor hormone receptor status. Even after such extensive investigation, there remains no consistent nor credible evidence that dietary acrylamide exposure increases the risk of any type of cancer in humans.

Initial concern about the possible carcinogenicity of acrylamide in humans arose primarily because of the observation that exposure to acrylamide led to increased tumor rates in mice and rat experiments in multiple target organs (Rice, 2005; Klaunig, 2008). The mouse experiments were in strains bred for hypersensitivity to induced tumors (lung tumors in A/J mice and skin tumors in Sencar mice) or were initiation-promotion studies

involving concomitant exposure to chemicals other than acrylamide (TPA and/or acetone in Sencar mice or Swiss-ICR mice). Increased incidence of skin tumors and lung tumors was observed in acrylamide-exposed mice, but both the selection of mouse strains and the short-term initiation-promotion experimental designs render these results of questionable relevance to human carcinogenicity (Rice, 2005; Klaunig, 2008).

Conventional rodent carcinogen bioassays have been conducted in Fischer 344 rats, with acrylamide exposure in drinking water over a 2-year period. In these feeding experiments, increased tumor rates were observed at several organ sites at the highest exposure levels (Rice, 2005; Klaunig, 2008). The elevated tumor rates among the rodents were observed only at exposure levels orders of magnitude higher (in terms of mg exposure per kilogram of body weight) than acrylamide levels to which humans are exposed, and no increased rates were observed at lower exposure levels, some of which exceeded known human exposure levels. In addition, even at the highest acrylamide exposure levels in the rat experiments, the tumor rates were not markedly elevated over the control rates at organ sites with increased incidence, particularly when only carcinomas were considered and nonmalignant tumors were excluded. It is noteworthy that any mechanism or mode of action to explain the observed increases in tumors at high exposure levels in rats exposed to acrylamide remains unclear (Klaunig, 2008).

The apparent discrepancy between the positive results of rodent studies and the negative results of epidemiologic studies has led to numerous comparative toxicokinetic studies of acrylamide. Acrylamide is biotransformed by the oxidative pathway into glycidamide, and toxicokinetic studies have largely focused on the production of this reactive epoxide, which is genotoxic and known to react with DNA. The toxicokinetic studies have demonstrated that acrylamide is detoxified quite efficiently in humans, and that the production of the potential carcinogen, glycidamide, is much lower in humans than in mice or rats (Fennell *et al.*, 2005, 2006; Fuhr *et al.*, 2006; Klaunig, 2008; Gargas *et al.*, 2009; Kopp and Dekant, 2009). Combined with the relatively low carcinogenic potency of acrylamide in rodents, these toxicokinetic studies indicate that the carcinogenicity of acrylamide in rodents is of questionable relevance to any potential acrylamide carcinogenicity in humans.

In humans, tobacco smoke makes a substantially larger contribution to acrylamide exposure compared with dietary acrylamide intake. The absence of an association between smoking and some of the cancers examined for acrylamide intake, particularly the absence of an increased risk of ovarian cancer among cigarette smokers (Vineis *et al.*, 2004) and the consistent inverse association observed between smoking and endometrial cancer

(Baron, 1996; Vineis *et al.*, 2004), two cancers for which Pelucchi *et al.* (2011b) call for further research, argues against an acrylamide effect for either of these cancers at the lower dietary acrylamide levels vis-à-vis cigarette smoking levels. Moreover, dietary acrylamide intake is likely to be more sporadic than cigarette exposure, and thus smoking not only leads to higher acrylamide levels but also produces continuous exposure more similar to that used in rat feeding experiments, which entail continuous exposure to acrylamide over a 2-year period. Thus, the absence of an association with smoking, in particular for ovarian and endometrial cancers, suggests that any association between the much lower, more sporadic dietary acrylamide exposure and increased risk of cancer is highly unlikely.

Because of the lack of credible epidemiologic evidence overall for cancer, and the inverse associations of dietary acrylamide intake levels and some cancers, Hogervorst *et al.* (2010) propose that if acrylamide causes cancer in humans at all, it may be through a hormonal mechanism. In view of the absence of empirical evidence that acrylamide exposure increases the risk of endometrial, ovarian, or breast cancer in women, however, the conjecture of a hormonal mechanism (or any other mechanism) of carcinogenesis is unwarranted at this time.

In summary, epidemiologic studies performed in various populations using different study designs and with wide ranges of estimated dietary acrylamide intake have failed to detect an increased risk of cancer, and they raise serious doubt regarding the validity of extrapolating from rodent studies suggestive of multiorgan effects to humans. In fact, the sporadically and slightly increased and decreased RRs reported in the numerous studies examined in this review strongly suggest the pattern one would expect to find for a true null association over the course of a series of trials. Therefore, continued epidemiologic investigation of acrylamide and the risk of cancer appears to be a misguided research priority in this era of increasingly limited research funding.

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Conflicts of interest

There are no conflicts of interest.

References

- Arab L (2010). Epidemiologic evidence on coffee and cancer. *Nutr Cancer* 62:271–283.
- Baron JA (1996). Beneficial effects of nicotine and cigarette smoking: the real, the possible and the spurious. *Br Med Bull* 52:58–73.

- Bjellaas T, Olesen PT, Frandsen H, Haugen M, Stolen LH, Paulsen JE, *et al.* (2007). Comparison of estimated dietary intake of acrylamide with hemoglobin adducts of acrylamide and glycidamide. *Toxicol Sci* **98**:110–117.
- Bravi F, Scotti L, Bosetti C, Gallus S, Negri E, La Vecchia C, *et al.* (2009). Coffee drinking and endometrial cancer risk: a meta-analysis of observational studies. *Am J Obstet Gynecol* **200**:130–135.
- Bull RJ, Robinson M, Laurie RD, Stoner GD, Greisiger E, Meier JR, *et al.* (1984). Carcinogenic effect of acrylamide in Sencar and A/J mice. *Cancer Res* **44**:107–111.
- Burley VJ, Greenwood DC, Hepworth SJ, Fraser LK, de Kok TM, van Breda SG, *et al.* (2010). Dietary acrylamide intake and risk of breast cancer in the UK women's cohort. *Br J Cancer* **103**:1749–1754.
- Collins JJ, Swaen GMH, Marsh GM, Utidjian HMD, Caporossi JC, Lucas LJ (1989). Mortality patterns among workers exposed to acrylamide. *J Occup Environ Med* **31**:614–617.
- Fennell TR, Sumner SCJ, Snyder RW, Burgess J, Spicer R, Bridson WE, *et al.* (2005). Metabolism and hemoglobin adduct formation of acrylamide in humans. *Toxicol Sci* **85**:447–459.
- Fennell TR, Sumner SCJ, Snyder RW, Burgess J, Friedman MA (2006). Kinetics of elimination of urinary metabolites of acrylamide in humans. *Toxicol Sci* **93**:256–267.
- Fuhr U, Boettcher MI, Kinzig-Schippers M, Weyer A, Jetter A, Lazar A, *et al.* (2006). Toxicokinetics of acrylamide in humans after ingestion of a defined dose in a test meal to improve risk assessment for acrylamide carcinogenicity. *Cancer Epidemiol Biomarkers Prev* **15**:266–271.
- Gargas ML, Kirman CR, Sweeney LM, Tardiff RG (2009). Acrylamide: consideration of species differences and nonlinear process in estimating risk and safety for human ingestion. *Food Chem Toxicol* **47**:760–768.
- Hagmar L, Wirfält E, Paulsson B, Törnqvist M (2005). Differences in hemoglobin adduct levels of acrylamide in the general population with respect to dietary intake, smoking habits and gender. *Mutat Res* **580**:157–165.
- Hirvonen T, Kontto J, Jestoi M, Valsta L, Peltonen K, Pietinen P, *et al.* (2010). Dietary acrylamide intake and the risk of cancer among Finnish male smokers. *Cancer Causes Control* **21**:2223–2229.
- Hogervorst JG, Schouten LJ, Konings EJM, Goldbohm RA, van den Brandt PA (2007). A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian and breast cancer. *Cancer Epidemiol Biomarkers Prev* **16**:2304–2313.
- Hogervorst JG, Schouten LJ, Konings EJM, Goldbohm RA, van den Brandt PA (2008a). Dietary acrylamide intake and the risk of renal cell, bladder, and prostate cancer. *Am J Clin Nutr* **87**:1428–1438.
- Hogervorst JG, Schouten LJ, Konings EJM, Goldbohm RA, van den Brandt PA (2008b). Dietary acrylamide intake is not associated with gastrointestinal cancer risk. *J Nutr* **138**:2229–2236.
- Hogervorst JG, Schouten LJ, Konings EJM, Goldbohm RA, van den Brandt PA (2009a). Lung cancer risk in relation to dietary acrylamide intake. *J Natl Cancer Inst* **101**:651–662.
- Hogervorst JG, Schouten LJ, Konings EJM, Goldbohm RA, van den Brandt PA (2009b). Dietary acrylamide intake and brain cancer risk. *Cancer Epidemiol Biomarkers Prev* **18**:1663–1666.
- Hogervorst JGF, Baars B-J, Schouten LJ, Konings EJM, Goldbohm RA, van den Brandt PA (2010). The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. *Crit Rev Toxicol* **40**:485–512.
- International Agency for Research on Cancer (1994). Acrylamide. *IARC monographs on the evaluation of carcinogenic risks to humans, volume 60, some industrial chemicals*. Lyon, France: IARC. pp. 389–433.
- Je Y, Hankinson SE, Tworoger SS, DeVivo I, Giovannucci E (2011). A prospective cohort study of coffee consumption and risk of endometrial cancer over a 26-year follow-up. *Cancer Epidemiol Biomarkers Prev* **20**:2487–2495.
- Johnson KA, Gorzinski SJ, Bodner KM, Campbell RA, Wolf CH, Friedman MA, *et al.* (1986). Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol Appl Pharmacol* **85**:154–168.
- Klaunig JE (2008). Acrylamide carcinogenicity. *J Agric Food Chem* **56**:5984–5988.
- Konings EJM, Hogervorst JGF, van Rooij L, Schouten LJ, Sizoo EA, van Egmond HP, *et al.* (2010). Validation of a database on acrylamide for use in epidemiologic studies. *Eur J Clin Nutr* **64**:534–540.
- Kopp EK, Dekant W (2009). Toxicokinetics of acrylamide in rats and humans following single oral administration of low doses. *Toxicol Appl Pharmacol* **235**:135–142.
- Larsson SC, Åkesson A, Bergkvist L, Wolk A (2009a). Dietary acrylamide intake and risk of colorectal cancer in a prospective cohort of men. *Eur J Cancer* **45**:513–516.
- Larsson SC, Åkesson A, Wolk A (2009b). Long-term dietary acrylamide intake and breast cancer risk in a prospective cohort of Swedish women. *Am J Epidemiol* **169**:376–381.
- Larsson SC, Åkesson A, Wolk A (2009c). Long-term dietary acrylamide intake and risk of epithelial ovarian cancer in a prospective cohort of Swedish women. *Cancer Epidemiol Biomarkers Prev* **18**:994–997.
- Larsson SC, Åkesson A, Wolk A (2009d). Dietary acrylamide intake and prostate cancer risk in a prospective cohort of Swedish men. *Cancer Epidemiol Biomarkers Prev* **18**:1939–1941.
- Larsson SC, Håkansson N, Åkesson A, Wolk A (2009e). Long-term dietary acrylamide intake and risk of endometrial cancer in a prospective cohort of Swedish women. *Int J Cancer* **124**:1196–1199.
- Lin Y, Lagergren J, Lu Y (2011). Dietary acrylamide intake and risk of esophageal cancer in a population-based case-control study in Sweden. *Int J Cancer* **128**:676–681.
- Marsh GM, Lucas LJ, Youk AO, Schall LC (1999). Mortality patterns among workers exposed to acrylamide: 1994 follow up. *Occup Environ Med* **56**:181–190.
- Marsh GM, Youk AO, Buchanich JM, Kant IJ, Swaen G (2007). Mortality patterns among workers exposed to acrylamide: updated follow up. *J Occup Environ Med* **49**:82–95.
- Mucci LA, Dickman PW, Steineck G, Adami HO, Augustsson K (2003a). Reply: dietary acrylamide and cancer risk: additional data on coffee. *Br J Cancer* **89**:775–776.
- Mucci LA, Dickman PW, Steineck G, Adami HO, Augustsson K (2003b). Dietary acrylamide and cancer of the large bowel, kidney, and bladder: absence of an association in a population-based study in Sweden. *Br J Cancer* **88**:84–89.
- Mucci LA, Lindblad P, Steineck G, Adami HO (2004). Dietary acrylamide and risk of renal cell cancer. *Int J Cancer* **109**:774–776.
- Mucci LA, Sandin S, Bälter K, Adami HO, Magnusson C, Weiderpass E (2005). Acrylamide intake and breast cancer risk in Swedish women. *JAMA* **293**:1326–1327.
- Mucci LA, Adami HO, Wolk A (2006). Prospective study of dietary acrylamide and risk of colorectal cancer among women. *Int J Cancer* **118**:169–173.
- Olesen PT, Olsen A, Frandsen H, Frederiksen K, Overvad K, Tjønneland A (2008). Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health study. *Int J Cancer* **122**:2094–2100.
- Pedersen GS, Hogervorst JGF, Schouten LJ, Konings EJM, Goldbohm RA, van den Brandt PA (2009). Dietary acrylamide intake and estrogen and progesterone receptor-defined postmenopausal breast cancer risk. *Breast Cancer Res Treat* **122**:199–210.
- Pelucchi C, Galeone C, Dal Maso L, Talamini R, Montella M, Ramazzotti V, *et al.* (2007). Dietary acrylamide and renal cell cancer. *Int J Cancer* **120**:1376–1377.
- Pelucchi CC, Galeone C, Levi F, Negri E, Franceschi S, Talamini R, *et al.* (2006). Dietary acrylamide and human cancer. *Int J Cancer* **118**:467–471.
- Pelucchi CC, Galeone C, Talamini R, Negri E, Polesel J, Serraino D, *et al.* (2011a). Dietary acrylamide and pancreatic cancer risk in an Italian case-control study. *Ann Oncol* **22**:910–915.
- Pelucchi CC, La Vecchia C, Bosetti C, Boyle P, Boffetta P (2011b). Exposure to acrylamide and human cancer – a review and meta-analysis of epidemiologic studies. *Ann Oncol* **22**:1487–1499.
- Rice JM (2005). The carcinogenicity of acrylamide. *Mutat Res* **580**:3–20.
- Schettgen T, Rossbach B, Kütting B, Letzel S, Drexler H, Angerer J (2004). Determination of haemoglobin adducts of acrylamide and glycidamide in smoking and non-smoking persons of the general population. *Int J Hyg Environ Health* **207**:531–539.
- Schouten LJ, Hogervorst JGF, Konings EJM, Goldbohm RA, van den Brandt PA (2009). Dietary acrylamide intake and the risk of head-neck and thyroid cancers: results from the Netherlands Cohort Study. *Am J Epidemiol* **170**:873–884.
- Sobel W, Bond GG, Parsons TW, Brenner FE (1986). Acrylamide cohort mortality study. *Br J Ind Med* **43**:785–788.
- Svensson K, Abramsson L, Becker W, Glynn A, Hellenäs K-E, Lind Y, *et al.* (2003). Dietary intake of acrylamide in Sweden. *Food Chem Toxicol* **41**:1581–1586.
- Swaen GMH, Haidar S, Burns CJ, Bodner K, Parsons T, Collins JJ, *et al.* (2007). Mortality study update of acrylamide workers. *Occup Environ Med* **64**:396–401.
- Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M (2000). Acrylamide: a cooking carcinogen? *Chem Res Toxicol* **13**:517–522.
- Van den Brandt PA, Goldbohm RA, van't Veer P, Volovics A, Hermus RJJ, Sturmans F (1990). A large-scale prospective cohort study on diet and cancer in the Netherlands. *J Clin Epidemiol* **43**:285–295.

- Vineis P, Alavanja M, Buffler P, Fontham E, Franceschi S, Gao YT, *et al.* (2004). Tobacco and cancer: recent epidemiological evidence. *J Natl Cancer Inst* **96**:99–106.
- Wilson KM, Bälter K, Adami HO, Grönberg H, Vikström AC, Paulsson B, *et al.* (2009a). Acrylamide exposure measured by food frequency questionnaire and hemoglobin adduct levels and prostate cancer risk in the Cancer of the Prostate in Sweden Study. *Int J Cancer* **124**: 2384–2390.
- Wilson KM, Mucci LA, Cho E, Hunter DJ, Chen WY, Willett WC (2009b). Dietary acrylamide intake and risk of premenopausal breast cancer. *Am J Epidemiol* **169**:954–961.
- Wilson KM, Vesper HW, Tocco P, Sampson L, Rosén J, Hellenäs KE, *et al.* (2009c). Validation of a food frequency questionnaire measurement of dietary acrylamide intake using hemoglobin adducts of acrylamide and glycidamide. *Cancer Causes Control* **20**:269–278.
- Wilson KM, Mucci LA, Rosner BA, Willett WC (2010). A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancer. *Cancer Epidemiol Biomarkers Prev* **19**:2503–2515.
- Wilson KM, Giovannucci E, Stampfer MJ, Mucci LA (2011). Dietary acrylamide and risk of prostate cancer. *Int J Cancer* [Aug 22 Epub ahead of print].
- Yu X, Bao Z, Zou J, Dong J (2011). Coffee consumption and risk of cancers: a meta-analysis of cohort studies. *BMC Cancer* **11**:96.

EXHIBIT C

Dietary Acrylamide Intake and the Risk of Lymphatic Malignancies: The Netherlands Cohort Study on Diet and Cancer

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Abstract

Background: Acrylamide, a probable human carcinogen, is present in many everyday foods. Since the finding of its presence in foods in 2002, epidemiological studies have found some suggestive associations between dietary acrylamide exposure and the risk of various cancers. The aim of this prospective study is to investigate for the first time the association between dietary acrylamide intake and the risk of several histological subtypes of lymphatic malignancies.

Methods: The Netherlands Cohort Study on diet and cancer includes 120,852 men and women followed-up since September 1986. The number of person years at risk was estimated by using a random sample of participants from the total cohort that was chosen at baseline ($n = 5,000$). Acrylamide intake was estimated from a food frequency questionnaire combined with acrylamide data for Dutch foods. Hazard ratios (HRs) were calculated for acrylamide intake as a continuous variable as well as in categories (quintiles and tertiles), for men and women separately and for never-smokers, using multivariable-adjusted Cox proportional hazards models.

Results: After 16.3 years of follow-up, 1,233 microscopically confirmed cases of lymphatic malignancies were available for multivariable-adjusted analysis. For multiple myeloma and follicular lymphoma, HRs for men were 1.14 (95% CI: 1.01, 1.27) and 1.28 (95% CI: 1.03, 1.61) per 10 μg acrylamide/day increment, respectively. For never-smoking men, the HR for multiple myeloma was 1.98 (95% CI: 1.38, 2.85). No associations were observed for women.

Conclusion: We found indications that acrylamide may increase the risk of multiple myeloma and follicular lymphoma in men. This is the first epidemiological study to investigate the association between dietary acrylamide intake and the risk of lymphatic malignancies, and more research into these observed associations is warranted.

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Introduction

In 2002, the scientific world was alarmed by the discovery of acrylamide in foods by the Swedish Food Authority. Acrylamide was classified as a proven rodent carcinogen and a probable human carcinogen by the International Agency for Research on Cancer in 1994, because of its carcinogenicity in rodents and because of the similarity between the way it is metabolized in rodents and in humans [1]. Several frequently consumed foods, such as French fries, cookies and coffee, contain high levels of acrylamide [2]. Acrylamide in food is formed in Maillard browning reactions, in which amino acids, asparagine in particular, react with reducing sugars during baking or other thermal processing at temperatures higher than 120 degrees

Celsius. Its formation depends on various cooking variables, particularly temperature and duration [3]. This causes large variations in the acrylamide content of different brands of the same food as well as among batches of a food of the same brand.

The mechanism by which acrylamide causes cancer in laboratory animals and by which it may cause cancer in humans is still unclear. Currently, the genotoxic action of glycidamide, which is an epoxide metabolite of acrylamide, is taken to be the mechanism of carcinogenic action in acrylamide risk assessments. Ample in vitro and in vivo animal studies have shown that acrylamide, mainly after metabolic conversion to glycidamide by the enzyme cytochrome P4502E1 (CYP2E1), causes chromosomal damage (aberrations, micronuclei, aneuploidy) and mutagenic effects [4]. However, the tissues with most DNA adducts or DNA

Table 1. Number of lymphatic malignancies in the Netherlands Cohort Study on diet and cancer (follow up: 16.3 years) according to the WHO classification.

Lymphatic malignancies	ICD-O-3 morphology codes	N ¹	N ²
T-cell, all			54
T-cell lymphoma	9701–9709, 9714	41	
Mucosis fungoides	9700	20	
B-cell, precursor lesions			
Acute lymphocytic leukemia	9836–9837	12	
Lymphoblastic lymphoma	9727–9728	8	
Burkitt's lymphoma	9687	4	
B-cell, other	9826, 9832–9833	2	
Malignant lymphomas, B-cell, mature neoplasms			
Diffuse large-cell lymphoma (DLCL)	9675, 9680, 9684	294	259
Follicular lymphoma (FL)	9690–9698	98	91
Waldenström macroglobulinemia and immunocytoma (WMI)	9671, 9761	90	89
Mantle cell lymphoma (MCL)	9673	63	56
Extranodal marginal B-cell lymphoma or MALT	9699	21	
Malignant lymphoma NOS	9590–9596	88	
Chronic lymphocytic leukemia	9670, 9823	224	200
Multiple myeloma	9731–9732, 9734	363	323
Hairy cell leukemia	9940	10	
Hodgkin lymphoma	9650–9669	37	
Total		1,375	1,233

Abbreviations: ICD-O-3, International Classification of Diseases for Oncology, 3rd edition; MALT, mucosa-associated lymphoid tissue; NOS, not otherwise specified.

¹N after exclusion of prevalent cases at baseline.

²N cases available for analyses, after exclusion of missing and inconsistent data. Only case numbers for subtypes with sufficient number of cases are given (so subgroups do not add up to 1,233).

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mutations do not consistently correspond to the tissues in which cancer occurred in the rat studies [5,6] and, more and more, other mechanisms of acrylamide carcinogenesis are being proposed [7,8]. Animal studies have shown positive dose-response relations between acrylamide intake through drinking water and cancer in multiple organs in mice and rats, such as the mammary glands, thyroid gland, testes and the uterus [4]. The presence of these mainly sex hormone-related cancers in animals, suggests a hormonal pathway [4,9,10], perhaps occurring in addition to genotoxic effects.

Since the finding of the presence of acrylamide in foods in 2002, epidemiological studies have evaluated various cancer endpoints in association with dietary acrylamide exposure of humans. A positive association for endometrial cancer was observed in two prospective cohort studies [11,12]. Both studies found a positive association for ovarian cancer as well, and in one of those studies this association was strongest in serous tumours [12]. Two studies, a cohort study and a nested case-control study, found a positive association between dietary acrylamide intake and the risk of estrogen receptor-positive breast cancer [13,14]. Further, a positive association was observed with renal cancer risk [15], and with oral cavity cancer risk in non-smoking women [16], both in a Dutch cohort study. In a Finnish prospective cohort study, a positive association was observed with lung cancer risk in smoking men [17].

Some epidemiological studies found indications for inverse associations with cancer risk, such as with lung cancer risk in women [18], prostate cancer risk in never-smoking men, bladder

cancer risk in women [15], and oro-and hypopharyngeal cancer risk in men [16].

Theoretically, every tissue in the human body, including lymphoid tissues, is a target for acrylamide carcinogenesis, because acrylamide is hydrophilic, and therefore it is able to diffuse passively throughout the whole body [19]. During the last decades, the occurrence of lymphatic malignancies has risen dramatically. This type of cancer is a heterogeneous group of malignancies derived from the T-cell and the B-cell development, and the most common types are multiple myeloma, diffuse large cell lymphoma and chronic lymphocytic leukemia [20]. Little is known about modifiable risk factors for these malignancies.

In the Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, no association was observed between dietary acrylamide intake and the risk of lymphomas in male smokers [17]. In this Finnish study, no analyses were done for histological subtypes of lymphatic malignancies, and therefore an association with a specific type of lymphatic malignancy might have been missed. Our study is the first to investigate the association between dietary acrylamide intake and the risk of several common subtypes of lymphatic malignancies.

Methods

Ethics Statement

By returning the completed questionnaire, the participants gave consent to participate in this study. The study protocol was approved by the Medical Ethics Committee of the University

Netherlands Cohort Study on diet and cancer

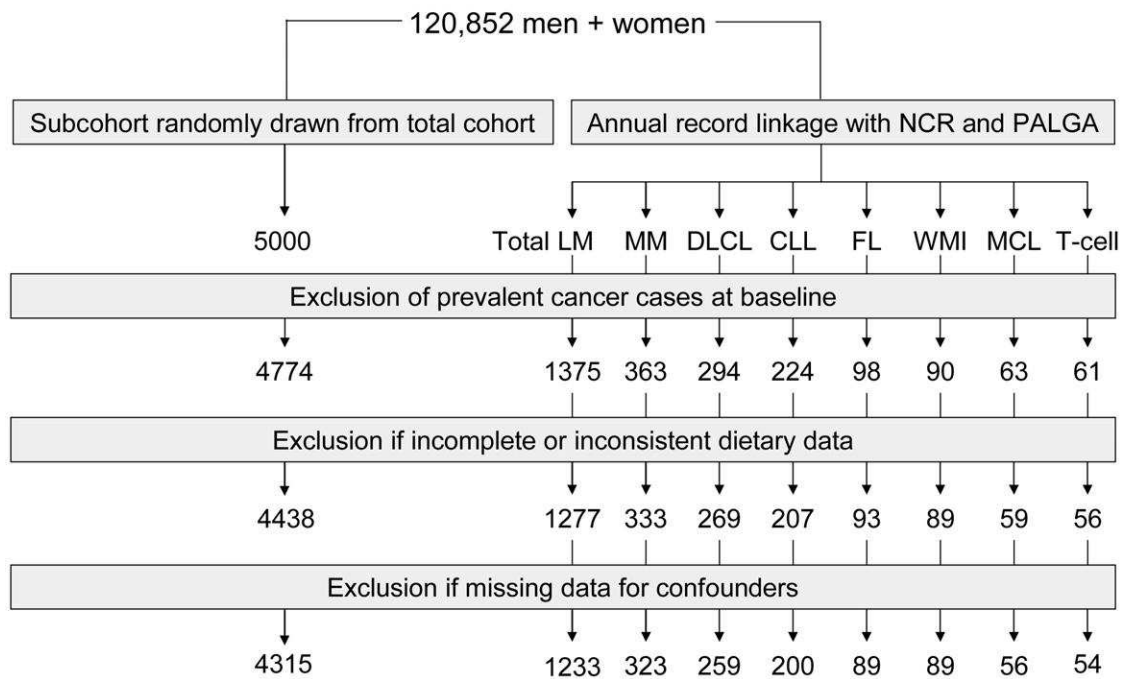


Figure 1. Flow diagram of subcohort members and cases used in the analysis. NCR = Netherlands Cancer Registry, PALGA = Netherlands Pathology Registry, LM = lymphatic malignancies, MM = multiple myeloma, DLCL = diffuse large cell lymphoma, CLL = chronic lymphocytic leukaemia, FL = follicular lymphoma, WMI = Waldenstrom macroglobulinemia and immunocytoma, MCL = mantle cell lymphoma, T-cell = T-cell lymphoma.

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Study Design and Population

In September 1986, 58,279 men and 62,573 women were enrolled in the Netherlands Cohort Study on diet and cancer (NLCS), a prospective cohort study [21]. All participants were at the age of 55–69 years at entry. Participants were selected through computerized municipal population registries.

Data processing and analysis were based on the case-cohort approach for efficiency reasons. The number of cancer cases (providing the numerator information for the estimation of cancer incidence rates) is the number of cases in the total cohort. The number of person-years at risk (providing the denominator information) was estimated by using a subcohort, a random sample of participants from the total cohort that was chosen at baseline ($n = 5,000$). Subcohort members were censored when they died, emigrated, reached the end of follow-up, or became a case, whichever came first.

Participants in this study are considered to be a case if they had a microscopically verified diagnosis of a lymphatic malignancy during the 16.3 year follow-up period. Incident cases in the total cohort were detected by annual computerized record linkages to the Netherlands Cancer Registry and the Netherlands Pathology Registry. The completeness of cancer follow-up through linkages with these registries is estimated to be at least 96% [22]. The histological subtype of the lymphatic malignancy was coded by the Netherlands Cancer Registry, using the International Classification of Diseases for Oncology, adapted for the Netherlands [23]. These codes were used to reclassify the lymphatic malignancies in categories according to the WHO classification of tumours of

hematopoietic and lymphoid tissues [24]. For cases that could not be assigned to a specific category, the summary of the pathology report of the Netherlands Pathology Registry was inspected, and, if possible, the case was assigned to a WHO category. Case numbers per histological subtype of lymphatic malignancies are given in table 1. The procedure is described elsewhere in more detail [25].

Cases and subcohort members were excluded from analysis if they reported cancer at baseline (except for skin cancer) or if their dietary data were incomplete or inconsistent. After 16.3 years of follow-up (from September 1986 through December 2002), there were 1375 cases of lymphatic malignancies, after exclusion of aforementioned cancer cases at baseline. Dietary data was complete and consistent for 1,277 cases, and 1,233 cases had complete data for covariables and were included in the analysis. Figure 1 shows the selection and exclusion steps that resulted in the numbers of cases and subcohort members that were available for analysis. Follow-up of the subcohort was nearly complete; only 1 male member of the subcohort was lost to follow-up.

Acrylamide Intake Assessment

Data on dietary habits and potential confounding variables were collected at baseline by means of the NLCS food frequency questionnaire (FFQ). The FFQ contains questions on 150 foods, measuring frequency and portion size for most.

To estimate acrylamide intake, we used a database with acrylamide concentrations in foods on the Dutch market, provided by the Dutch Food and Consumer Product Safety Authority. In 2002, this authority analyzed the levels of acrylamide in various Dutch foods, such as bread, French fries, pastry, cake and Dutch spiced cake [26]. In 2005, the authority analyzed several foods to specifically accommodate the estimation of acrylamide intake of

Table 2. Characteristics of cases and subcohort members according to sex in the Netherlands Cohort Study on diet and cancer, 1986–2002.

Variable ¹	Subcohort	CLL	DLCL	MM	FL	WMI	MCL	T-cell
Men								
N	2,191	139	165	172	44	54	41	36
<i>Dietary variables</i>								
Acrylamide intake (µg/d)	23 (12)	21 (11)	23 (13)	25 (13)	26 (16)	26 (17)	22 (10)	22 (11)
Acrylamide intake BW (µg/d per kg bw)	0.29 (0.16)	0.28 (0.15)	0.29 (0.17)	0.33 (0.18)	0.34 (0.21)	0.33 (0.21)	0.27 (0.13)	0.29 (0.15)
Coffee (g/d)	578 (290)	576 (304)	561 (265)	547 (233)	588 (295)	545 (209)	570 (296)	601 (283)
Dutch spiced cake (g/d)	4.1 (8.6)	3.2 (6.6)	4.2 (8.4)	5.5 (9.7)	8.9 (14.2)	6.4 (13.6)	3.8 (7.4)	3.1 (7.7)
Cookies (g/d)	14 (11)	14 (9)	13 (11)	16 (18)	12 (10)	15 (10)	16 (9)	17 (10)
Potato chips (g/d)	0.47 (1.72)	0.30 (1.01)	0.59 (3.16)	0.62 (2.54)	0.21 (0.57)	0.35 (0.71)	0.59 (1.84)	0.33 (1.02)
French fries (g/d)	7.2 (15.4)	6.3 (12.8)	7.7 (19.9)	9.4 (17.9)	4.8 (8.0)	9.1 (17.1)	4.6 (11.7)	5.4 (10.5)
Total energy intake (kcal/d)	2,162 (510)	2,208 (509)	2,133 (486)	2,194 (541)	2,164 (568)	2,239 (489)	2,171 (395)	2,180 (375)
Carbohydrate (g/d)	227 (66)	229 (61)	226 (61)	232 (65)	224 (56)	238 (66)	220 (44)	232 (48)
Saturated fat (g/d)	37 (12)	39 (13)	36 (12)	37 (12)	35 (12)	37 (11)	36 (10)	37 (10)
Trans unsaturated fatty acid (g/d)	3.3 (1.7)	3.6 (1.7)	3.5 (2.0)	3.6 (1.8)	2.9 (1.3)	3.4 (1.6)	3.1 (1.5)	3.2 (1.4)
Total fatty acids (g/d)	87 (27)	91 (27)	86 (25)	90 (29)	89 (34)	87 (25)	89 (23)	88 (22)
Mono unsaturated fat (g/d)	35 (12)	38 (12)	35 (11)	37 (13)	36 (16)	36 (10)	35 (10)	36 (10)
Poly unsaturated fat (g/d)	20 (10)	19 (8)	19 (9)	21 (10)	22 (14)	19 (9)	23 (11)	20 (8)
Fiber (g/d)	29 (9)	29 (9)	28 (9)	30 (9)	29 (9)	31 (8)	30 (9)	30 (8)
Alcohol (g/d)	15 (17)	14 (16)	14 (17)	12 (13)	12 (13)	19 (22)	17 (15)	12 (13)
Niacin (g/d)	15 (5)	16 (5)	15 (4)	15 (5)	16 (6)	16 (4)	15 (4)	16 (5)
<i>Non-dietary variables</i>								
Age (y)	61.3 (4.2)	62.1 (4.1)	62.0 (4.0)	61.8 (3.9)	61.1 (4.4)	61.8 (4.1)	62.0 (4.4)	61.1 (4.2)
BMI (kg/m ²)	25.0 (2.6)	25.0 (2.4)	25.0 (2.4)	25.2 (2.7)	24.8 (2.7)	25.4 (2.3)	25.2 (2.0)	24.6 (2.6)
Height (m)	1.76 (0.07)	1.77 (0.06)	1.78 (0.07)	1.76 (0.07)	1.78 (0.08)	1.76 (0.07)	1.80 (0.06)	1.77 (0.08)
Non-occupational physical activity (min/d)	80 (68)	84 (84)	79 (68)	80 (62)	107 (96)	66 (52)	82 (85)	79 (72)
Family history of HM (%)	2.5	5.0	4.9	2.9	9.1	3.7	7.3	0.0
<i>Cigarette smoking</i>								
Never-smokers (%)	12.7	15.1	11.5	13.4	11.4	9.3	19.5	13.9
Former smokers (%)	51.6	55.4	47.9	59.3	38.6	59.3	58.5	41.7
Current smokers (%)	35.7	29.5	40.6	27.3	50.0	31.5	22.0	44.4
Smoking quantity (n cig./d) ²	17 (11)	15 (11)	16 (10)	15 (10)	20 (12)	18 (15)	16 (11)	15 (8)
Smoking duration (y) ²	34 (12)	34 (12)	34 (12)	32 (13)	35 (10)	32 (13)	33 (13)	36 (11)
<i>Education</i>								
Primary school (%)	25.0	24.6	25.8	22.2	27.9	24.1	17.5	8.3
Lower vocational school (%)	20.7	18.1	19.6	26.3	20.9	9.3	20.0	22.2
Intermediate vocational school (%)	35.6	40.6	31.9	33.3	32.6	31.5	40.0	50.0
Higher vocational school (%)	18.7	16.7	22.7	18.1	18.6	35.2	22.5	19.4
Women								
N	2247	68	104	161	49	35	18	20
<i>Dietary variables</i>								
Acrylamide intake (µg/d)	21 (12)	20 (11)	21 (11)	21 (13)	23 (18)	23 (15)	20 (14)	27 (19)
Acrylamide intake BW (µg/d per kg bw)	0.32 (0.19)	0.29 (0.17)	0.31 (0.17)	0.30 (0.19)	0.34 (0.26)	0.33 (0.20)	0.31 (0.25)	0.40 (0.30)
Coffee (g/d)	497 (245)	494 (241)	503 (202)	511 (234)	515 (258)	471 (285)	458 (271)	494 (172)
Dutch spiced cake (g/d)	5.7 (9.4)	4.9 (8.4)	5.4 (8.9)	5.2 (9.8)	7.9 (16.3)	8.2 (11.8)	6.1 (11.6)	10.0 (14.8)
Cookies (g/d)	14 (11)	14 (9)	14 (14)	15 (12)	13 (9)	15 (11)	14 (9)	15 (11)
Potato chips (g/d)	0.40 (1.93)	0.62 (3.15)	0.19 (0.76)	0.16 (0.46)	0.16 (0.52)	0.27 (0.94)	0.11 (0.28)	0.43 (1.08)

Table 2. Cont.

Variable ¹	Subcohort	CLL	DLCL	MM	FL	WMI	MCL	T-cell
French fries (g/d)	4.0 (8.8)	1.7 (4.6)	3.1 (7.7)	4.0 (12.5)	2.8 (8.5)	3.4 (6.0)	1.4 (3.4)	5.7 (11.1)
Total energy intake (kcal)	1,683 (397)	1,762 (463)	1,634 (339)	1,711 (402)	1,747 (384)	1,708 (302)	1,628 (370)	1,878 (468)
Carbohydrate (g/d)	179 (48)	190 (58)	174 (45)	184 (45)	183 (49)	184 (35)	165 (40)	205 (63)
Saturated fat (g/d)	30 (10)	31 (11)	30 (9)	30 (10)	32 (9)	29 (7)	29 (9)	35 (11)
Trans unsaturated fatty acid (g/d)	2.5 (1.2)	2.3 (0.9)	2.4 (0.9)	2.6 (1.3)	2.5 (0.9)	2.3 (0.9)	2.2 (0.9)	3.1 (1.4)
Total fatty acids (g/d)	69 (22)	71 (28)	68 (18)	70 (23)	70 (16)	69 (16)	70 (21)	77 (22)
Mono unsaturated fat (g/d)	28 (9)	29 (12)	27 (7)	28 (10)	28 (7)	28 (7)	29 (10)	31 (9)
Poly unsaturated fat (g/d)	15 (7)	15 (10)	15 (7)	15 (8)	14 (7)	17 (6)	16 (5)	16 (7)
Fiber (g/d)	25 (7)	26 (8)	26 (7)	27 (8)	26 (6)	27 (7)	26 (5)	26 (6)
Alcohol (g/d)	5.9 (9.5)	5.5 (9.6)	3.7 (7.2)	4.6 (7.7)	5.9 (10.7)	5.5 (8.9)	6.6 (8.5)	5.7 (8.6)
Niacin (g/d)	12 (3)	13 (5)	12 (3)	13 (4)	13 (3)	12 (3)	13 (3)	13 (3)
<i>Non-dietary variables</i>								
Age (y)	61.4 (4.3)	62.6 (4.0)	62.0 (4.6)	62.4 (4.2)	61.9 (4.3)	62.0 (4.2)	61.6 (4.4)	60.3 (4.3)
BMI (kg/m ²)	25.1 (3.6)	25.1 (4.3)	24.6 (3.3)	25.6 (3.6)	15.2 (3.6)	25.0 (3.6)	23.9 (2.7)	24.9 (3.3)
Height (m)	1.65 (0.06)	1.67 (0.07)	1.66 (0.07)	1.66 (0.06)	1.66 (0.05)	1.67 (0.05)	1.66 (0.08)	1.65 (0.07)
Non-occupational physical activity (min/d)	64 (53)	67 (59)	62 (54)	70 (62)	62 (44)	76 (71)	69 (71)	52 (31)
Family history of HM (%)	3.2	2.9	4.8	5.6	6.1	0.0	11.1	5.0
<i>Cigarette smoking</i>								
Never-smokers (%)	58.4	66.2	65.4	67.7	57.1	57.1	50.0	65.0
Former smokers (%)	20.6	20.6	17.3	21.1	14.2	25.7	27.8	20.0
Current smokers (%)	21.0	13.2	17.3	11.2	28.6	17.1	22.2	15.0
Smoking quantity (n cig./d) ²	11 (8)	15 (12)	12 (8)	11 (8)	11(7)	11 (5)	12 (7)	8 (11)
Smoking duration (y) ²	28 (13)	27 (14)	29 (11)	26 (12)	30 (11)	30 (11)	23 (14)	24 (12)
<i>Education</i>								
Primary school (%)	33.5	29.4	35.0	34.4	27.1	34.3	22.2	35.0
Lower vocational school (%)	23.2	25.0	19.4	25.0	20.8	17.1	22.2	10.0
Intermediate vocational school (%)	34.5	38.2	39.8	34.4	43.8	40.0	44.4	45.0
Higher vocational school (%)	8.8	7.4	5.8	6.3	8.3	8.6	11.1	10.0

MM = multiple myeloma; DLCL = diffuse large cell lymphoma; CLL = chronic lymphocytic leukemia; FL = follicular lymphoma; WMI = Waldenström macroglobulinemia and immunocytoma; MCL = mantle cell lymphoma; T-cell = T-cell lymphomas; BW = bodyweight; HM = hematological malignancies.

¹Mean (standard deviation) or percentage.

²Among former or current smokers.

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the NLCS cohort, such as various types of bread, specific types of cookies, cake and pastry, chocolate and chocolate milk, nuts and salty snacks, peanut butter and coffee. Acrylamide was measured in types of cookies which were known to be eaten most frequently by a population comparable with the NLCS, as was known from the development phase of the questionnaire. Bread was sampled and analyzed again in 2005, because the quantitation limit of the analytic method had decreased from 30 ppb in 2002 to 15 ppb in 2005. This change offered the opportunity to more accurately estimate the acrylamide intake via bread. To determine the acrylamide level for each food, the mean values of the acrylamide measurements per food were used, or, in case the concentrations were lower than the quantitation limit, a value one-half the quantitation limit. This database was recently validated in a study comparing estimated acrylamide content (using acrylamide data from the database) and measured acrylamide content of duplicate 24-hour diets [27]. This rendered a correlation coefficient of 0.82, which indicates that it is feasible to make a sound rank ordering of

the acrylamide intake via a 24-hour meal using the mean acrylamide levels for individual foods in the database.

The acrylamide intake for each participant in the study was estimated by multiplying the acrylamide level of each food with the frequency of consumption and the portion size of that food, and summing up these values across all foods.

Statistical Analysis

Acrylamide is included in the multivariable-adjusted models as a continuous variable per 10 µg per day intake of acrylamide as well as a categorical variable, to be able to investigate the dose-response relationship, where possible. For acrylamide to be modeled as a categorical variable, we required at least 100 cases for quintile categories in subgroup analyses or 60 cases for tertile categories. In case there were less than 60, but more than 20 cases in a subgroup, we analyzed acrylamide as a continuous variable only. Following these criteria, we thus analyzed multiple myeloma, diffuse large cell lymphoma, chronic lymphocytic leukemia, follicular lymphoma, Waldenström macroglobulinemia and immunocytoma in

Table 3. Association between dietary acrylamide intake and the risk of multiple myeloma, diffuse large cell lymphoma, and chronic lymphocytic leukemia according to sex and smoking status; the Netherlands Cohort Study on diet and cancer, 1986–2002.¹

	Acrylamide intake (per 10 µg/d)	Q1/T1	Q2	Q3/T2	Q4	Q5/T3	P for trend
Multiple myeloma							
All men							
Cases/py	170/28,981	32/5,656	20/5,661	35/5,899	34/5,833	49/5,933	
HR (CI 95%) ²	1.15 (1.04–1.27)	1.00 (ref)	0.63 (0.36–1.13)	1.10 (0.67–1.82)	1.09 (0.66–1.82)	1.51 (0.94–2.41)	0.01
HR (CI 95%) ³	1.14 (1.01–1.27)	1.00 (ref)	0.65 (0.36–1.16)	1.14 (0.67–1.94)	1.14 (0.67–1.94)	1.54 (0.92–2.58)	0.02
Never-smoking men							
Cases/py	23/3933						
HR (CI 95%) ²	1.59 (1.24–2.03)	⁴	⁴	⁴	⁴	⁴	⁴
HR (CI 95%) ³	1.98 (1.38–2.85)	⁴	⁴	⁴	⁴	⁴	⁴
All women							
Cases/py	153/32,296	25/6,305	41/6,586	34/6,286	23/6,630	30/6,489	
HR (CI 95%) ²	0.99 (0.85–1.16)	1.00 (ref)	1.64 (0.98–2.74)	1.46 (0.86–2.49)	0.93 (0.52–1.67)	1.21 (0.70–2.09)	0.66
HR (CI 95%) ³	0.92 (0.77–1.11)	1.00 (ref)	1.46 (0.85–2.49)	1.19 (0.67–2.12)	0.73 (0.39–1.37)	0.93 (0.50–1.73)	0.22
Never-smoking women							
Cases/py	102/19,005	13/4,058	32/3,866	19/3,414	16/3,903	22/3,765	
HR (CI 95%) ²	1.08 (0.89–1.30)	1.00 (ref)	2.61 (1.34–5.08)	1.85 (0.90–3.83)	1.33 (0.63–2.81)	1.86 (0.92–3.76)	0.71
HR (CI 95%) ³	1.01 (0.80–1.26)	1.00 (ref)	2.37 (1.19–4.73)	1.54 (0.72–3.29)	1.03 (0.46–2.31)	1.43 (0.68–3.02)	0.61
Diffuse large cell lymphoma							
All men							
Cases/py	159/28,981	32/5,656	28/5,661	35/5,899	34/5,833	30/5,933	
HR (CI 95%) ²	1.02 (0.90–1.17)	1.00 (ref)	0.89 (0.53–1.50)	1.12 (0.68–1.84)	1.12 (0.67–1.85)	0.93 (0.56–1.56)	0.94
HR (CI 95%) ³	1.04 (0.91–1.20)	1.00 (ref)	0.93 (0.54–1.59)	1.23 (0.74–2.04)	1.26 (0.74–2.17)	1.06 (0.61–1.86)	0.73
Never-smoking men							
Cases/py	19/3,933	⁴	⁴	⁴	⁴	⁴	⁴
All women							
Cases/py	100/32,296	17/6,305	17/6,586	24/6,286	24/6,630	18/6,489	
HR (CI 95%) ²	0.98 (0.84–1.14)	1.00 (ref)	0.99 (0.50–1.98)	1.49 (0.78–2.84)	1.41 (0.73–2.69)	1.06 (0.54–2.09)	0.80
HR (CI 95%) ³	1.02 (0.85–1.24)	1.00 (ref)	1.05 (0.51–2.15)	1.71 (0.87–3.36)	1.72 (0.84–3.50)	1.38 (0.63–3.02)	0.43
Never-smoking women							
Cases/py	64/19,005	17/6,063		28/6,306		19/6,636	
HR (CI 95%) ²	1.00 (0.81–1.22)	1.00 (ref)		1.67 (0.91–3.07)		1.05 (0.54–2.03)	0.73
HR (CI 95%) ³	1.06 (0.83–1.36)	1.00 (ref)		1.79 (0.94–3.38)		1.27 (0.61–2.66)	0.94
Chronic lymphocytic leukemia							
All men ³							
Cases/py	134/28,981						
HR (CI 95%) ²	0.94 (0.81–1.09)	⁵	⁵	⁵	⁵	⁵	⁵
HR (CI 95%) ³	0.88 (0.74–1.04)	⁵	⁵	⁵	⁵	⁵	⁵
Never-smoking men							
Cases/py	21/3,933						
HR (CI 95%) ²	1.04 (0.76–1.40)	⁴	⁴	⁴	⁴	⁴	⁴
HR (CI 95%) ³	1.12 (0.82–1.54)	⁴	⁴	⁴	⁴	⁴	⁴
All women ³							
Cases/py	66/32,296	24/9,743		19/11,168		23/11,385	
HR (CI 95%) ²	0.99 (0.71–1.10)	1.00 (ref)		0.74 (0.40–1.37)		0.85 (0.47–1.53)	0.74
HR (CI 95%) ³	0.83 (0.64–1.09)	1.00 (ref)		0.74 (0.38–1.43)		0.81 (0.42–1.57)	0.70
Never-smoking women							

Table 3. Cont.

	Acrylamide intake (per 10 µg/d)	Q1/T1	Q2	Q3/T2	Q4	Q5/T3	P for trend
cases/py	45/19,005						
HR (CI 95%) ²	0.97 (0.75–1.25)	⁴	4	4	4	4	4
HR (CI 95%) ³	0.95 (0.70–1.30)	⁴	4	4	4	4	4

¹HR = hazard ratio; CI = Confidence Interval; py = person years; Q = quintile; T = tertile. The number of cases and person-years are the numbers that resulted after listwise deletion of observations with missing values for the selected confounders. HRs were calculated by using Cox proportional hazards analysis.

²Adjusted for age and sex.

³Adjusted for age (years), sex, height (per 10 cm), education level, fiber (g/d), total fatty acids (g/d), trans unsaturated fatty acid (g/d), mono unsaturated fat (g/d), poly unsaturated fat (g/d), carbohydrates (g/d) and niacin (mg/d).

⁴Insufficient number of cases for analyses with tertiles (N>60 required) or with acrylamide as a continuous variable (N>20 required).

⁵Proportional hazards assumption not met; therefore results not presented.

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models with acrylamide as a continuous and categorical variable, and mantle cell lymphoma, and T-cell lymphoma in models with acrylamide as a continuous variable only. The numbers of other subtypes were too small for separate analyses.

Besides age and sex, the following variables were tested to assess potential confounding, based on the literature: body mass index (BMI), height, non-occupational physical activity, education level, vegetable and fruit intake, intake of several nutrients (such as fat and saturated fat, trans fatty acids, carbohydrates, fiber and niacin), alcohol consumption, smoking, reproductive factors (in women only; age at menarche, menopause and first childbirth, parity, and use of oral contraceptives and postmenopausal

hormone treatment), and immune system-related diseases self-reported at baseline (such as hepatitis, tuberculosis and rheumatoid arthritis). Those variables that modified the hazard ratio of acrylamide (with a unit of 27 µg/day: the interval between the 10th and 90th percentile of acrylamide intake in the subcohort) by 10% or more for any endpoint were used in the final multivariable-adjusted model, which was subsequently applied to all endpoints. Some variables were tested for interaction on the basis of their ability to modify the activity of the enzyme CYP2E1. The variables concerned are age, BMI, diabetes, physical activity, and alcohol consumption [28,29].

Table 4. Association between continuously modeled dietary acrylamide intake (per 10 µg/d) and the risk of follicular lymphoma and Waldenström macroglobulinemia and immunocytoma (WMI); the Netherlands Cohort Study on diet and cancer, 1986–2002.¹

	Follicular lymphoma	Waldenström macroglobulinemia and immunocytoma	Mantle cell lymphoma	T-cell lymphomas
All men				
Cases/py	42/28,981	54/28,981	38/28,981	35/28,981
HR per 10 µg/d (CI 95%) ¹	1.20 (0.98–1.47)	1.18 (0.96–1.44)	0.96 (0.76–1.21)	0.94 (0.70–1.25)
HR per 10 µg/d (CI 95%) ²	1.28 (1.03–1.61)	1.18 (0.93–1.50)	1.06 (0.85–1.31)	0.94 (0.68–1.29)
Never-smoking men				
Cases/py	5/3,933	5/3,933	7/3,933	5/3,933
HR per 10 µg/d (CI 95%) ¹	³	³	³	³
HR per 10 µg/d (CI 95%) ²	³	³	³	³
All women				
Cases/py	47/32,296	35/32,296	18/32,296	19/32,296
HR per 10 µg/d (CI 95%) ¹	1.12 (0.83–1.51)	1.13 (0.85–1.50)	³	³
HR per 10 µg/d (CI 95%) ²	1.12 (0.80–1.57)	1.21 (0.88–1.66)	³	³
Never-smoking women				
Cases/py	27/19,005	20/19,005	9/19,005	12/19,005
HR per 10 µg/d (CI 95%) ¹	1.23 (0.91–1.67)	1.14 (0.78–1.68)	³	³
HR per 10 µg/d (CI 95%) ²	1.18 (0.82–1.71)	1.27 (0.84–1.91)	³	³

HR = hazard ratio; CI = confidence interval; py = person years. The number of cases and person-years are the numbers that resulted after listwise deletion of observations with missing values for the selected confounders. HRs were calculated by using Cox proportional hazards analysis.

¹Adjusted for age and sex.

²Adjusted for age (years), sex, height (per 10 cm), education level, fiber (g/d), total fatty acids (g/d), trans unsaturated fatty acid (g/d), mono unsaturated fat (g/d), poly unsaturated fat (g/d), carbohydrates (g/d) and niacin (mg/d).

³Insufficient number of cases for analyses with acrylamide as a continuous variable (N>20 required).

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Table 5. Acrylamide hazard ratios (and 95% CI) of multiple myeloma, diffuse large cell lymphoma and chronic lymphatic leukemia in **men** in strata of several covariables and *p* values for interaction: the Netherlands Cohort Study on diet and cancer, 1986–2002.

Interaction variable	MM			DLCL			CLL		
	N cases/ person- years	HR per 10 µg AA/d	<i>P</i> for inter- action	N cases/ person- years	HR per 10 µg AA/d	<i>P</i> for interaction	N cases/ person- years	HR per 10 µg AA/d	<i>P</i> for Interaction
Smoking status									
Never	23/3,933	1.92 (1.34–2.75)	0.02	19/3,933	1.03 (0.67–1.57)	0.05	21/3,933	1.06 (0.80–1.40)	0.45
Former	101/15,124	0.99 (0.82–1.19)		76/15,124	1.17 (0.97–1.42)		72/15,124	0.69 (0.53–0.90)	
Current	46/9,925	1.18 (0.96–1.44)		64/9,925	0.90 (0.74–1.09)		41/9,925	1.04 (0.79–1.36)	
Current smoking									
Smoking quantity (n cig./d)									
0	23/3,933	1.92 (1.34–2.75)	0.03	19/3,933	1.03 (0.67–1.57)	0.64	21/3,933	1.06 (0.80–1.40)	0.77
0 to <15	60/9,445	1.11 (0.92–1.34)		61/9,445	1.04 (0.85–1.28)		52/9,445	0.80 (0.62–1.03)	
≥15	82/13,935	1.04 (0.84–1.29)		73/13,935	1.13 (0.88–1.44)		52/13,935	0.86 (0.59–1.24)	
Smoking duration (y)									
0	23/3,933	1.92 (1.34–2.75)	0.02	19/3,933	1.03 (0.67–1.57)	0.61	21/3,933	1.06 (0.80–1.40)	0.30
0 to <30	56/8,513	1.00 (0.81–1.23)		48/8,513	1.04 (0.83–1.31)		39/8,513	0.60 (0.40–0.89)	
>30	89/16,117	1.12 (0.95–1.31)		91/16,117	1.06 (0.88–1.28)		73/16,117	0.97 (0.76–1.23)	
Age									
Ever and never-smokers									
55–59	58/11,922	1.03 (0.83–1.27)	0.48	50/11,922	0.93 (0.65–1.31)	0.07	43/11,922	0.96 (0.76–1.22)	0.37
60–64	64/10,059	1.23 (0.97–1.55)		62/10,059	1.24 (0.99–1.55)		50/10,059	0.81 (0.57–1.15)	
65–69	48/7,000	1.19 (0.99–1.43)		47/7,000	0.84 (0.60–1.20)		41/7,000	0.83 (0.56–1.24)	
Never-smokers									
55–59	7/1,602	²	²	³			6/1,602	²	²
60–64	9/1,285	²					5/1,285	²	
65–69	7/1,045	²					10/1,045	0.92 (0.42–2.01)	
BMI (kg/m ²)									
Ever and never-smokers									
<20	2/520	²	²	2/520	²	²	4/520	²	²
≥20–25	82/14,885	1.26 (1.10–1.44)		76/14,885	1.08 (0.90–1.29)		59/14,885	0.85 (0.66–1.10)	
>25	85/13,380	0.88 (0.70–1.09)		79/13,380	1.03 (0.82–1.31)		70/13,380	0.91 (0.73–1.14)	
Never-smokers									
<20	0/64	²	²	³			1/64	²	²
≥20–25	15/2,130	2.09 (1.43–3.05)					13/2,130	1.00 (0.59–1.70)	
>25	7/1,706	²					7/1,706	²	
Diabetes									
Ever and never-smokers									
No	162/28,192	1.14 (1.02–1.29)	²	156/28,192	1.05 (0.91–1.21)	²	128/28,192	0.88 (0.74–1.04)	²
Yes	8/790	²		3/790	²		6/790	²	
Never-smokers									
No	22/3,840	1.94 (1.34–2.82)	²	³			20/3,840	1.14 (0.82–1.59)	²
Yes	1/93	²					1/93	²	
Physical activity (min/d)									
Ever and never-smokers									
<30	26/4,503	1.11 (0.85–1.45)	0.85	38/4,503	0.84 (0.61–1.16)	0.40	29/4,503	0.72 (0.50–1.04)	0.57

Table 5. Cont.

Interaction variable	MM			DLCL			CLL		
	N cases/ person- years	HR per 10 µg AA/d	P for inter- action	N cases/ person- years	HR per 10 µg AA/d	P for interaction	N cases/ person- years	HR per 10 µg AA/d	P for Interaction
30–60	52/9,341	1.21 (0.94–1.56)		44/9,341	1.07 (0.84–1.36)		38/9,341	0.90 (0.65–1.24)	
61–90	38/5,467	0.99 (0.75–1.31)		275,467	1.09 (0.67–1.76)		25/5,467	0.84 (0.50–1.41)	
>90	53/9,203	1.14 (0.94–1.39)		48/9,203	1.13 (0.90–1.41)		40/9,203	0.98 (0.74–1.31)	
Never-smokers									
<30	3/656	²	²	³			4/656	²	²
30–60	9/1,116	²					6/1,116	²	
61–90	5/873	²					3/873	²	
>90	6/1,181	²					8/1,181	²	
Alcohol intake (g/d)									
Ever and never-smokers									
0	14/4,004	0.78 (0.34–1.77)	0.53	25/4,004	1.12 (0.93–1.34)	0.72	12/4,004	1.08 (0.69–1.71)	0.56
>0–5	46/6,084	1.29 (1.02–1.64)		34/6,084	1.01 (0.62–1.66)		35/6,084	0.92 (0.61–1.41)	
>5	109/18,553	1.14 (0.98–1.33)		99/18,553	0.99 (0.82–1.20)		85/18,553	0.81 (0.65–1.01)	
Never-smokers									
0	2/874	²	²	³			2/874	²	²
>0–5	11/1,122	1.81 (1.17–2.80)					6/1,122	²	
>5	10/1,921	2.28 (1.28–4.06)					13/1,921	0.96 (0.56–1.64)	

Abbreviations: HR = hazard ratio; CI = confidence interval; AA/d = acrylamide per day; MM = multiple myeloma; CLL = chronic lymphatic leukemia; DLCL = diffuse large cell lymphoma.

¹Adjusted for age, sex, height (per 10 cm), education level, fiber (g/d), total fatty acids (g/d), trans unsaturated fatty acid (g/d), mono unsaturated fat (g/d), poly unsaturated fat (g/d), carbohydrates (g/d) and niacin (mg/d).

²Insufficient number of cases for analyzing interaction.

³This subgroup was not analyzed at all, due to insufficient number of cases (n<20).

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To test the proportional hazards assumption, models were run using scaled Schoenfeld residuals. Hazard ratios and corresponding 95% confidence intervals were obtained by performing Cox proportional hazards regression using STATA software (version 9; Stata Corp, College Station, TX) per subtype of lymphatic malignancies for men and women separately. Standard errors (SEs) were estimated by using the robust Huber-White sandwich estimator to account for additional variance introduced by sampling the subcohort from the cohort.

Smokers have on average three to four times higher levels of acrylamide-hemoglobin adducts (which is a marker of internal dose of acrylamide) than non-smokers. [30]. To preclude confounding through the exposure of cigarette smoke, subgroup analyses were performed for never-smokers.

Effect modification of the association between acrylamide intake and cancer by other variables was tested using Wald chi-square tests (by means of the testparm option in Stata). We thus tested whether there were any statistically significant differences in the beta coefficients of acrylamide between the strata of the interaction variable.

To assess whether observed associations could be attributed to acrylamide intake or to the foods that contain the acrylamide, in separate analyses, acrylamide hazard ratios were adjusted for the five foods explaining most of the variance in acrylamide intake in our population, namely Dutch spiced cake, coffee, cookies, potato crisps and French fries.

All analyses were repeated without the first two years of follow-up to investigate protopathic bias.

Results

The most outstanding differences between subcohort members and cases were observed for physical activity and family history between male subcohort members and male follicular lymphoma cases, and family history between female subcohort members and female mantle cell lymphoma cases (see table 2).

As described elsewhere, coffee was overall the biggest source of acrylamide, but Dutch spiced cake was mainly responsible for the variation in acrylamide intake, and next most responsible were coffee, French fries, potato crisps, and cookies [31].

The hazard ratios for endpoints with more than 100 cases are presented in table 3. For multiple myeloma, there was an increased HR for the continuous acrylamide variable in all men (smokers and non-smokers combined) (HR per 10 µg acrylamide/day: 1.14; 95% CI: 1.01, 1.27), and a trend across the quintiles of acrylamide intake (*p* for trend = 0.02). For never-smoking men, the HR for the continuous acrylamide intake was increased as well (HR per 10 µg acrylamide/day: 1.98; 95% CI: 1.38, 2.85). Unfortunately, the limited number of cases in this subgroup prohibited investigation of the dose-response relationship over categories of acrylamide intake. No association with multiple

Table 6. Acrylamide hazard ratios (and 95% CI) of multiple myeloma, diffuse large cell lymphoma and chronic lymphatic leukemia in **women** in strata of several covariables and *p* values for interaction: the Netherlands Cohort Study on diet and cancer, 1986–2002.

	MM			DLCL			CLL		
Interaction variable	N cases/ person- years	HR per 10 µg AA/d	<i>P</i> for interaction	N cases/ person- years	HR per 10 µg AA/d	<i>P</i> for interaction	N cases/ person-years	HR per 10 µg AA/d	<i>P</i> for interaction
Smoking status									
Never	102/19,005	1.02 (0.80–1.26)	0.24	64/19,005	1.06 (0.83–1.36)	0.36	45/19,005	0.93 (0.68–1.27)	²
Former	33/6,716	0.74 (0.49–1.12)		18/6,716	1.29 (0.86–1.91)		13/6,716	0.72 (0.41–1.28)	
Current	18/6,574	0.67 (0.31–1.44)		18/6,574	0.49 (0.19–1.24)		8/6,574	²	
Current smoking									
Smoking quantity (n cig./d)									
0	102/19,005	1.01 (0.80–1.26)	0.23	64/19,005	1.06 (0.83–1.36)	0.83	45/19,005	0.93 (0.68–1.27)	²
0 to <15	37/8,238	0.63 (0.39–1.02)		24/8,238	1.06 (0.72–1.55)		11/8,238	0.79 (0.44–1.41)	
≥15	14/4,501	0.94 (0.49–1.83)		12/4,501	0.83 (0.50–1.36)		9/4,501	²	
Smoking duration (y)									
0	102/19,005	1.01 (0.80–1.26)	0.18	64/19,005	1.06 (0.83–1.36)	0.83	45/19,005	0.93 (0.68–1.27)	²
0 to <30	28/6,405	0.66 (0.39–1.12)		15/6,405	1.05 (0.60–1.87)		9/6,405	²	
≥30	22/6,530	0.79 (0.42–1.49)		21/6,530	0.98 (0.64–1.50)		11/6,530	0.66 (0.31–1.43)	
Age									
Ever and never-smokers									
55–59	43/13,090	0.89 (0.68–1.16)	0.66	36/13,090	1.03 (0.76–1.38)	0.14	17/13,090	0.44 (0.16–1.23)	0.27
60–64	57/10,768	1.00 (0.74–1.36)		29/10,768	1.41 (0.96–2.07)		23/10,768	0.96 (0.62–1.49)	
65–69	53/8,437	0.88 (0.60–1.30)		35/8,437	0.77 (0.56–1.06)		26/8,437	0.96 (0.70–1.32)	
Never-smokers									
55–59	27/6,852	0.90 (0.67–1.22)	0.87	20/6,852	1.05 (0.74–1.51)	0.22	6/6,852	²	²
60–64	38/6,424	0.99 (0.65–1.52)		18/6,424	1.35 (0.82–2.22)		17/6,424	0.80 (0.45–1.42)	
65–69	37/5,729	1.13 (0.73–1.74)		26/5,729	0.82 (0.56–1.21)		22/5,729	1.01 (0.69–1.49)	
BMI (kg/m ²)									
Ever and never-smokers									
<20	4/1,520	²	²	4/1,520	²	²	9/1,520	²	²
≥20–25	66/16,362	0.83 (0.61–1.14)		51/16,362	1.03 (0.79–1.35)		30/16,362	0.67 (0.41–1.09)	
>25	80/14,279	1.04 (0.81–1.32)		45/14,279	1.04 (0.78–1.38)		27/14,279	0.99 (0.68–1.45)	
Never-smokers									
<20	1/658	²	²	1/658	²	²	5/658	²	²
≥20–25	42/9,189	0.91 (0.60–1.37)		33/9,189	1.03 (0.71–1.51)		19/9,189	0.81 (0.43–1.52)	
>25	58/9,119	1.11 (0.85–1.45)		30/9,119	1.04 (0.76–1.43)		21/9,119	1.12 (0.75–1.69)	
Diabetes									
Ever and never-smokers									
No	148/31,252	0.90 (0.75–1.07)	²	97/31,252	1.03 (0.85–1.25)	²	63/31,252	0.84 (0.64–1.11)	²
Yes	5/1,044	²		3/1,044	²		3/1,044	²	
Never-smokers									
No	98/18,264	0.96 (0.77–1.19)	²	63/18,264	1.06 (0.82–1.37)	²	42/18,264	0.97 (0.71–1.34)	²
Yes	4/742	²		1/742	²		3/742	²	
Physical activity (min/d)									
Ever and never-smokers									

Table 6. Cont.

	MM			DLCL			CLL		
Interaction variable	N cases/ person-years	HR per 10 µg AA/d	P for interaction	N cases/ person-years	HR per 10 µg AA/d	P for interaction	N cases/ person-years	HR per 10 µg AA/d	P for interaction
<30	34/7,170	0.54 (0.35–0.83)	0.42	26/7,170	1.16 (0.86–1.58)	0.47	15/7,170	0.74 (0.45–1.22)	0.16
30–60	52/9,977	1.07 (0.79–1.45)		30/9,977	1.12 (0.82–1.53)		22/9,977	0.90 (0.57–1.43)	
61–90	32/7,236	1.01 (0.71–1.43)		21/7,236	0.95 (0.59–1.53)		16/7,236	0.75 (0.39–1.44)	
>90	31/7,089	1.10 (0.69–1.77)		21/7,089	0.78 (0.52–1.16)		13/7,089	1.05 (0.58–1.89)	
Never-smokers									
<30	23/4,493	0.49 (0.25–0.95)	0.26	15/4,493	1.24 (0.82–1.86)	0.83	12/4,493	0.79 (0.43–1.45)	²
30–60	36/6,215	1.36 (0.97–1.90)		21/6,215	1.17 (0.81–1.71)		15/6,215	1.10 (0.68–1.77)	
61–90	21/3,784	1.06 (0.71–1.57)		15/3,784	0.70 (0.31–1.57)		11/3,784	1.10 (0.58–2.09)	
>90	20/3,948	1.03 (0.51–2.09)		12/3,948	0.92 (0.54–1.55)		7/3,948	²	
Alcohol intake (g/d)									
Ever and never-smokers									
0	40/9,793	0.92 (0.67–1.28)	0.81	35/9,793	1.01 (0.72–1.41)	0.62	21/9,793	0.89 (0.56–1.39)	0.69
>0–5	65/11,351	0.95 (0.69–1.32)		45/11,351	0.98 (0.72–1.35)		21/11,351	0.60 (0.37–0.97)	
>5	39/9,812	0.94 (0.66–1.34)		18/9,812	1.15 (0.84–1.59)		21/9,812	1.00 (0.62–1.63)	
Never-smokers									
0	28/6,975	0.88 (0.59–1.31)	0.78	21/6,975	0.76 (0.46–1.26)	²	18/6,975	0.91 (0.52–1.57)	²
>0–5	51/7,410	1.13 (0.79–1.61)		33/7,410	1.21 (0.84–1.74)		16/7,410	0.62 (0.36–1.06)	
>5	17/3,692	1.05 (0.62–1.77)		8/3,692	²		8/3,692	²	

Abbreviations: HR = hazard ratio; CI = confidence interval; AA/d = acrylamide per day; MM = multiple myeloma; CLL = chronic lymphatic leukemia; DLCL = diffuse large cell lymphoma.

¹Adjusted for age, sex, height (per 10 cm), education level, fiber (g/d), total fatty acids (g/d), trans unsaturated fatty acid (g/d), mono unsaturated fat (g/d), poly unsaturated fat (g/d), carbohydrates (g/d) and niacin (mg/d).

²Insufficient number of cases.

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myeloma was observed in women, except for an increased HR in the 2nd quintile in never-smoking women.

Acrylamide was not associated with diffuse large cell lymphoma in any of the subgroups of men, women, or never-smoking men and women.

We observed decreased risks of chronic lymphocytic leukemia in both men and women for acrylamide as a continuous variable. In the subgroup of men, the proportional hazards assumption was violated in the quintile analysis. When we split the follow-up time at 2 years, at 8 years, or at 5 and 10 years, no clear associations between acrylamide intake and CLL risk in men were observed. For instance, in the first 8 years of follow-up, the hazard ratios for tertiles of acrylamide intake were 0.85 (95% CI: 0.45–1.63) and 0.80 (95% CI: 0.41–1.55) for the 2nd and 3rd tertile, respectively, with a p-trend of 0.50 (*n* = 53). In the last 8.3 years the corresponding values were 1.10 (95% CI: 0.61–1.99) and 0.67 (0.34–1.31), with a p-trend of 0.19 (*n* = 81). No association was seen in never-smokers.

Table 4 shows the hazard ratios for the continuous acrylamide variable for endpoints with less than 100 cases. The HR for acrylamide as a continuous variable for follicular lymphoma in all men was increased (HR per 10 µg acrylamide/day: 1.28; 95% CI: 1.03, 1.61), but not in all or never-smoking women.

We did not observe associations between acrylamide intake and the risk of Waldenström macroglobulinemia and immunocytoma in men or in women, or mantle cell lymphoma or T-cell-

lymphoma in men. There were too few women in these latter two groups for meaningful analyses.

The results of the analyses of interactions between acrylamide and possible CYP2E1-influencing variables are presented in table 5 (men) and 6 (women) for multiple myeloma, diffuse large cell lymphoma and chronic lymphocytic leukemia. The numbers for other subtypes were too small for analysis of interaction.

In the analyses of multiple myeloma, smoking status modified the acrylamide-associated risk in men. Never-smokers had a higher acrylamide-associated risk of multiple myeloma (HR per 10 µg acrylamide/day: 1.92 (95% CI: 1.34, 2.75; *p* for interaction = 0.02)) than former or current smokers, which was also reflected by the interaction with smoking quantity and duration. Although there was no statistically significant interaction with alcohol, we observed an increased acrylamide-associated risk of multiple myeloma in never-smoking men with the highest (>5 g/day) intake of alcohol (HR 2.28 (95% CI: 1.28, 4.06). For diffuse large cell lymphoma, smoking status modified the acrylamide-associated risk in men, with former smokers having the highest acrylamide-associated risk (HR per 10 µg acrylamide/day: 1.17 (95% CI: 0.97, 1.42); *p* for interaction = 0.05), but not in women (*p* for interaction = 0.36).

For chronic lymphocytic leukemia, there was no interaction between acrylamide intake and any of the CYP2E1-influencing variables.

In sensitivity analyses, the associations between acrylamide and the endpoints of lymphatic malignancies did not change after

exclusion of cases diagnosed during the first two years of follow-up. Although the HRs of acrylamide intake were slightly attenuated after additional adjustment for coffee, and increased after adjustment for Dutch spiced cake, the results did not change importantly. The results also did not change when adjusted for the other foods that contribute most to the variance in acrylamide intake, which were cookies, French fries and potato crisps. All of these foods were themselves not associated with the risk of lymphatic malignancies (results not shown).

At the time of the analyses described in this paper, our data on lymphatic malignancies were not classified according to the InterLymph classification [32]. We have checked how our classification of the cases corresponds to the InterLymph classification for the types of lymphatic malignancies that we observed associations with acrylamide for (multiple myeloma and follicular lymphoma in men). There were no (multiple myeloma) or minor differences ($n = 1$ for follicular lymphoma) between the two classifications. When we left out the male case that was a follicular lymphoma case in our dataset, but that would have been a chronic/small lymphocytic leukemia/lymphoma case in the InterLymph classification, the hazard ratios for acrylamide were virtually unchanged. A major difference between the WHO classification and the InterLymph classification lies in the way lymphomas with morphology code M9675 are classified. In the WHO classification, they are grouped under diffuse large cell lymphoma, but not all M9675 lymphomas are large cell lymphomas and some are T-cell lymphomas. We have repeated the analysis of the diffuse large cell lymphoma group excluding the M9675 codes ($n = 27$ men, 12 women) (which then renders the group of diffuse large B-cell lymphomas according to the InterLymph recommendations) and the results were essentially unchanged.

Discussion

This prospective cohort study is, to our knowledge, the first epidemiological study to investigate the association between dietary acrylamide intake and the risk of specific histological subtypes of lymphatic malignancies. Because of this, the results of this study are challenging, but should be interpreted cautiously. We observed a positive association for multiple myeloma in all men and never-smoking men, and for follicular lymphoma in all men.

In the Finnish ATBC Study, no association was observed between dietary acrylamide intake and the risk of lymphomas in male smokers [17]. In that study, no analyses were done for histological subtypes of lymphatic malignancies, and therefore an association with a specific type of lymphatic malignancy might have been obscured. In addition, when studying the link between dietary acrylamide intake and cancer risk, it is better to study non-smokers as a subgroup, because cigarette smoke is a much more important source of acrylamide than diet is and it might therefore blur the association between acrylamide through diet and cancer risk.

Possible risk factors for lymphatic malignancies, such as height, overweight, hormones and nutrients, have shown contradictory results in epidemiological studies [20,25]. Although there is thus no strong epidemiological evidence for risk factors for lymphatic malignancies, in the present study we checked the confounding potential of a broad range of possible risk factors for lymphatic malignancies and cancer in general. Human immunodeficiency virus (HIV) infection has been associated with an increased risk of lymphatic malignancies [20]. Data on the prevalence of HIV in our study population was not available, but the prevalence was

likely low, considering the age segment of our population. We were able to check for other immune system-related diseases, such as asthma and tuberculosis, but these diseases were not found to be confounders for the association between acrylamide intake and the risk of lymphatic malignancies.

The present study has some limitations that should be discussed. The associations between dietary acrylamide intake and multiple myeloma in never-smoking men, and the association for follicular lymphoma in all men were based on analyses with a small number of cases. This makes it likely that some of the observed associations are spurious. Therefore, these results should be interpreted cautiously. Moreover, we analyzed associations in many subtypes of lymphatic malignancies and for several subgroups within each subtype, which makes it likely that chance findings have occurred. The same applies to the subgroup analyses that were done to investigate interaction with CYP2E1-influencing variables. However, the indications for possible interaction with smoking and alcohol are intriguing, although based on analyses with a small number of cases, as both smoking and alcohol intake were inversely associated with the glycidamide to acrylamide hemoglobin adduct ratio in a cross-sectional population study [33].

In addition, this study has some limitations regarding acrylamide intake assessment. Firstly, within foods, acrylamide levels vary greatly, which leads to non-differential misclassification of acrylamide intake when assigning a single mean acrylamide value to a food, which most likely biases risk estimates towards null. This means that true risks, if any, are probably greater than the risks presented here. Moreover, a recent study has shown that it is feasible to make a sound rank ordering of the acrylamide intake via a 24-hour meal using the mean acrylamide levels used in the NLCS study for individual foods. [27]. Secondly, the acrylamide values in our food database were derived from foods that were sampled in 2002 and 2005. They may not be completely representative of the foods that were on the market in 1986. Thirdly, the FFQ did not provide information on which foods were prepared at home and how this was done. Both aspects too will have resulted in some non-differential misclassification of the intake, which will then most likely have led to underestimation of the true risks. Despite the fact that the use of an FFQ has limitations for the assessment of dietary acrylamide exposure, as is extensively discussed elsewhere [34], it is the only feasible way to assess dietary acrylamide intake over a long period of time in a large study population.

Although we have no direct data for acrylamide itself, the reproducibility and validity of the NLCS FFQ for acrylamide can to some extent be derived from nutrients that are correlated to acrylamide, namely carbohydrates and dietary fiber. The decline of the correlation between the baseline questionnaire and the questionnaire administered after 5 years of follow-up was 0.07 on average among the nutrients that were tested. This indicates that, although the questionnaire was administered only once, it characterizes long-term food intake for over a period of at least five years [35]. As for validity, the correlation coefficients between the FFQ and a dietary record method were 0.77 for carbohydrates and 0.74 for fiber. For the food groups potatoes, bread, and cakes and cookies, Spearman correlation coefficients were 0.74, 0.80 and 0.65, respectively [36].

The large study size and the prospective nature of this NLCS are important strengths of this study. Selection bias is unlikely to occur, as the follow-up of the participants was complete. Due to the prospective design of the study, recall bias is absent. In addition, we were able to obtain a dietary acrylamide intake estimate representative for the Dutch study population, by estimating acrylamide levels in several batches of various Dutch

food products that were specific for the population under study. The large study size enabled us to study specific histological subtypes of lymphatic malignancies that differ in their etiology and, as indicated by this study, may differ regarding their association with dietary acrylamide intake.

Recent analyses within the NLCs, the Nurses' Health Study, and a Danish cohort study [11,12,13,14] showed a positive association for endometrial, ovarian, and estrogen receptor-positive breast cancer, suggesting that disturbance of sex hormonal balances may be a mechanism of acrylamide carcinogenesis, which can also be suggested based on the rat carcinogenicity assays [7,8]. Although it cannot be concluded from the present study, hormonal imbalances might be a mechanism of acrylamide carcinogenesis for lymphatic malignancies as well. Men have a higher incidence of lymphatic malignancies than women [20], but the reasons for this higher incidence are not known. Sex hormones have been shown to influence the immune system [37] and may thus be at the basis of this observed difference. Estrogen receptor expression in lymphocytes suggests that estrogen bioavailability may be relevant to the pathogenesis of lymphomas [38]. For multiple myeloma, studies investigated the mechanism of anti-estrogens (AEs), and showed that AEs inhibit cell cycle progression of malignant multiple myeloma cells and/or induce apoptosis in these cells [39]. Other studies suggest that hormone-related and reproductive factors are involved in the etiology of lymphatic malignancies [40,41,42], and in a different way for men and women [43], but the results are inconsistent.

This is the first epidemiological study to investigate the association between dietary acrylamide intake and the risk of lymphatic malignancies. It provides indications that acrylamide may increase the risk of multiple myeloma and follicular lymphoma, but on the basis of the present study alone, we cannot conclude whether these results reflect true biological effects or are chance findings. We recommend that this possible modifiable risk factor for lymphatic malignancies is investigated in other prospective studies.

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Author Contributions

Conceived and designed the experiments: JGFH IJS RAG HCS PAvdB. Analyzed the data: MLB JGFH. Wrote the paper: MLB. Drafted the manuscript or revised it critically for important intellectual content: MLB JGFH IJS RAG HCS PAvdB. Final approval of the version to be published: MLB JGFH IJS RAG HCS PAvdB.

References

- (1994) Monographs on the evaluation of carcinogen risk to human: some industrial chemicals. Lyon, France: International Agency for Research on Cancer.
- Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M (2002) Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem* 50: 4998–5006.
- Mottram DS, Wedzicha BL, Dodson AT (2002) Acrylamide is formed in the Maillard reaction. *Nature* 419: 448–449.
- Besaratinia A, Pfeiffer GP (2007) A review of mechanisms of acrylamide carcinogenicity. *Carcinogenesis* 28: 519–528.
- Segerback D, Callemann CJ, Schroeder JL, Costa LG, Faustman EM (1995) Formation of N-7-(2-carbamoyl-2-hydroxyethyl)guanine in DNA of the mouse and the rat following intraperitoneal administration of [¹⁴C]acrylamide. *Carcinogenesis* 16: 1161–1165.
- Mei N, McDaniel LP, Dobrovolsky VN, Guo X, Shaddock JG, et al. (2010) The genotoxicity of acrylamide and glycidamide in big blue rats. *Toxicol Sci* 115: 412–421.
- Dourson M, Hertzberg R, Allen B, Haber L, Parker A, et al. (2008) Evidence-based dose-response assessment for thyroid tumorigenesis from acrylamide. *Regul Toxicol Pharmacol* 52: 264–289.
- Haber LT, Maier A, Kroner OL, Kohrman MJ (2009) Evaluation of human relevance and mode of action for tunica vaginalis mesotheliomas resulting from oral exposure to acrylamide. *Regul Toxicol Pharmacol* 53: 134–149.
- Klaunig JE (2008) Acrylamide carcinogenicity. *J Agric Food Chem* 56: 5984–5988.
- Hogervorst JG, Baars BJ, Schouten LJ, Konings EJ, Goldbohm RA, et al. (2010) The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. *Crit Rev Toxicol* 40: 485–512.
- Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2007) A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev* 16: 2304–2313.
- Wilson KM, Mucci LA, Rosner BA, Willett WC (2010) A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancers. *Cancer Epidemiol Biomarkers Prev* 19: 2503–2515.
- Olesen PT, Olsen A, Frandsen H, Frederiksen K, Overvad K, et al. (2008) Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study. *Int J Cancer* 122: 2094–2100.
- Pedersen GS, Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, et al. (2010) Dietary acrylamide intake and estrogen and progesterone receptor-defined postmenopausal breast cancer risk. *Breast Cancer Res Treat* 122: 199–210.
- Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2008) Dietary acrylamide intake and the risk of renal cell, bladder, and prostate cancer. *Am J Clin Nutr* 87: 1428–1438.
- Schouten LJ, Hogervorst JG, Konings EJ, Goldbohm RA, van den Brandt PA (2009) Dietary acrylamide intake and the risk of head-neck and thyroid cancers: results from the Netherlands Cohort Study. *Am J Epidemiol* 170: 873–884.
- Hirvonen T, Kontto J, Jestoi M, Valsta L, Peltonen K, et al. (2010) Dietary acrylamide intake and the risk of cancer among Finnish male smokers. *Cancer Causes Control* 21: 2223–2229.
- Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2009) Lung cancer risk in relation to dietary acrylamide intake. *J Natl Cancer Inst* 101: 651–662.
- Friedman M (2003) Chemistry, biochemistry, and safety of acrylamide. A review. *J Agric Food Chem* 51: 4504–4526.
- Hartge P, Wang SS, Bracci PM, Devesa SS, Holly EA (2006) Non-Hodgkin lymphoma. In: Schottenfeld D, Fraumeni JF Jr, editors. *Cancer Epidemiology and Prevention*. third ed. New York: Oxford University Press.
- van den Brandt PA, Goldbohm RA, van't Veer P, Volovics A, Hermus RJ, et al. (1990) A large-scale prospective cohort study on diet and cancer in The Netherlands. *J Clin Epidemiol* 43: 285–295.
- Goldbohm RA, van den Brandt PA, Dorant E (1994) Estimation of the coverage of Dutch municipalities by cancer registries and PALGA based on hospital discharge data. *Tijdschr Soc Gezondheidsz* 72: 80–84.
- (1993) International classification of diseases for oncology, adapted for the Netherlands. Utrecht, the Netherlands: Integrale kankercentra.
- Jaffe E, Harris N., Stein H., et al (2001) World Health Organization classification of tumours. Pathology and genetics of tumours of hematopoietic and lymphoid tissues. Lyon, France: IARC Press.
- Pylypchuk RD, Schouten LJ, Goldbohm RA, Schouten HC, van den Brandt PA (2009) Body mass index, height, and risk of lymphatic malignancies: a prospective cohort study. *Am J Epidemiol* 170: 297–307.
- Konings EJ, Baars AJ, van Klaveren JD, Spanjer MC, Rensen PM, et al. (2003) Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. *Food Chem Toxicol* 41: 1569–1579.
- Konings EJ, Hogervorst JG, van Rooij L, Schouten LJ, Sizoo EA, et al. (2010) Validation of a database on acrylamide for use in epidemiological studies. *Eur J Clin Nutr* 64: 534–540.
- Ghanayem BI, Witt KL, Kissling GE, Tice RR, Recio L (2005) Absence of acrylamide-induced genotoxicity in CYP2E1-null mice: evidence consistent with a glycidamide-mediated effect. *Mutat Res* 578: 284–297.
- Lamy E, Volkel Y, Roos PH, Kassie F, Mersch-Sundermann V (2008) Ethanol enhanced the genotoxicity of acrylamide in human, metabolically competent HepG2 cells by CYP2E1 induction and glutathione depletion. *Int J Hyg Environ Health* 211: 74–81.

30. Schettgen T, Rossbach B, Kutting B, Letzel S, Drexler H, et al. (2004) Determination of haemoglobin adducts of acrylamide and glycidamide in smoking and non-smoking persons of the general population. *Int J Hyg Environ Health* 207: 531–539.
31. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2007) A Prospective Study of Dietary Acrylamide Intake and the Risk of Endometrial, Ovarian, and Breast Cancer. *Cancer Epidemiol Biomarkers Prev* 16: 2304–2313.
32. Morton LM, Turner JJ, Cerhan JR, Linet MS, Treseler PA, et al. (2007) Proposed classification of lymphoid neoplasms for epidemiologic research from the Pathology Working Group of the International Lymphoma Epidemiology Consortium (InterLymph). *Blood* 110: 695–708.
33. Vesper HW, Slimani N, Hallmans G, Tjonneland A, Agudo A, et al. (2008) Cross-sectional study on acrylamide hemoglobin adducts in subpopulations from the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. *J Agric Food Chem* 56: 6046–6053.
34. Konings E, Hogervorst J.G.F., Schouten L.J., Brandt van den P.A., (2006) Assessing exposure levels of acrylamide. In: Skog A, Alexander, J., editor. *Acrylamide and other hazardous compounds in heat-treated foods*. Cambridge, United Kingdom: Woodhead Publishing Limited. 214–225.
35. Goldbohm RA, van 't Veer P, van den Brandt PA, van 't Hof MA, Brants HA, et al. (1995) Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. *Eur J Clin Nutr* 49: 420–429.
36. Goldbohm RA, van den Brandt PA, Brants HA, van't Veer P, Al M, et al. (1994) Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. *Eur J Clin Nutr* 48: 253–265.
37. Bouman A, Heineman MJ, Faas MM (2005) Sex hormones and the immune response in humans. *Hum Reprod Update* 11: 411–423.
38. Stygar D, Westlund P, Eriksson H, Sahlin L (2006) Identification of wild type and variants of oestrogen receptors in polymorphonuclear and mononuclear leucocytes. *Clin Endocrinol (Oxf)* 64: 74–81.
39. Renoir JM, Boudier C, Seguin A, Marsaud V, Sola B (2008) Antioestrogen-mediated cell cycle arrest and apoptosis induction in breast cancer and multiple myeloma cells. *J Mol Endocrinol* 40: 101–112.
40. Morton LM, Wang SS, Richesson DA, Schatzkin A, Hollenbeck AR, et al. (2009) Reproductive factors, exogenous hormone use and risk of lymphoid neoplasms among women in the National Institutes of Health-AARP Diet and Health Study Cohort. *Int J Cancer* 124: 2737–2743.
41. Skibola CF, Bracci PM, Halperin E, Nieters A, Hubbard A, et al. (2008) Polymorphisms in the estrogen receptor 1 and vitamin C and matrix metalloproteinase gene families are associated with susceptibility to lymphoma. *PLoS One* 3: e2816.
42. Skibola CF, Bracci PM, Paynter RA, Forrest MS, Agana L, et al. (2005) Polymorphisms and haplotypes in the cytochrome P450 17A1, prolactin, and catechol-O-methyltransferase genes and non-Hodgkin lymphoma risk. *Cancer Epidemiol Biomarkers Prev* 14: 2391–2401.
43. Everaus H, Hein M, Zilmer K (1993) Possible imbalance of the immuno-hormonal axis in multiple myeloma. *Leuk Lymphoma* 11: 453–458.

Null Results in Brief

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Dietary Acrylamide Intake and Risk of Renal Cell Carcinoma in Two Large Prospective Cohorts

Rebecca E. Graff^{1,2}, Eunyoung Cho^{3,4,5}, Mark A. Preston⁶, Alejandro Sanchez⁷, Lorelei A. Mucci^{1,3}, and Kathryn M. Wilson^{1,3}

Abstract

Background: Accumulating evidence suggests that dietary acrylamide intake is not associated with the risk of most cancers in humans. However, a meta-analysis of five epidemiologic studies found a suggestion of an increased risk of kidney cancer with higher dietary acrylamide intake.

Methods: We investigated this association in the prospective Health Professionals Follow-up Study (HPFS; 1986–2014) and Nurses' Health Study (NHS; 1980–2014) cohorts. Dietary acrylamide intake was calculated on the basis of 46 acrylamide-containing foods reported on food frequency questionnaires completed every 4 years. The associations with the incidence of total and fatal renal cell carcinoma (RCC; $n = 292/84$ HPFS, $n = 337/87$ NHS) during more than two

decades of follow-up were assessed using Cox proportional hazards models adjusting for potential confounders.

Results: There was no association between cumulative average or baseline acrylamide intake and the risk of total or fatal RCC risk in men or women. Acrylamide intake was also not associated with RCC risk among never-smokers, nor was it associated with the risk of clear cell RCC.

Conclusions: Dietary acrylamide was not associated with risk of RCC in two long-term prospective cohorts with repeated measures of dietary intake.

Impact: This analysis of RCC adds to the body of evidence that dietary acrylamide is not an important cancer risk factor in humans. *Cancer Epidemiol Biomarkers Prev*; 27(8); 979–82. ©2018 AACR.

Introduction

Acrylamide has been designated a probable human carcinogen based largely on evidence from animal studies (1). Among its most abundant dietary sources are coffee, French fries, potato chips, cereal, and other foods made from grains. Cigarette smoke and occupational settings are also exposure sources. Epidemiologic evidence suggests that dietary acrylamide is not associated with risk of most cancers (2). However, a meta-analysis of five studies of dietary acrylamide intake and kidney cancer found a borderline significant association, with a stronger association when restricting to two prospective studies (2).

We studied this association in two long-term prospective studies, the Health Professionals Follow-up Study (HPFS) and Nurses' Health Study (NHS), which collect repeated measures of diet over time.

Materials and Methods

Study populations

The HPFS is a cohort of 51,529 male health professionals, ages 40–75 at baseline in 1986. The NHS is a cohort of 121,701 female nurses, ages 30–55 at baseline in 1976. The cohorts have been described (3). These analyses began follow-up in 1986 and 1980 for the HPFS and NHS respectively, when participants completed initial semiquantitative food frequency questionnaires (FFQs). Participants with complete FFQs and no prior cancer at baseline, 47,797 men and 88,767 women, formed the study population. Their dietary intakes of total acrylamide and 46 high-acrylamide foods (among them breads, baked goods, cereal, potatoes, and coffee) were measured every 4 years (4). We used cumulative average intakes as primary exposures and adjusted for energy intake using the residual method (5).

Cancers were identified by self-report or participants' next-of-kin on biennial questionnaires, and confirmed by medical records. Renal cell carcinoma (RCC) cases included pathologically confirmed clear cell, papillary, chromophobe, collecting duct, spindle cell/sarcomatoid, and unclassified RCC (6). Deaths were identified via family and the National Death Index. Follow-up for mortality is >98% complete.

Statistical analysis

Cox models were used to estimate HR and 95% confidence intervals (CIs) for associations between intake of acrylamide and high-acrylamide foods and total and fatal RCC risk. Follow-up ended at the earliest of RCC diagnosis, death date, or end of follow-up (January 2014 HPFS and June 2014 NHS). Analyses were conducted separately in the two cohorts, and meta-analyzed using random effects models. Models were adjusted for the variables described in the table footnotes.

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Results

In the HPFS, 292 cases of RCC were diagnosed during a median follow-up of 27.2 years. In the NHS, 337 cases were diagnosed during a median follow-up of 33.9 years. At baseline, those with the highest dietary acrylamide were younger, had less hypertension, and were more likely smokers than those with lower intakes (Table 1).

Multivariable models showed no association between dietary acrylamide and RCC risk in men (top vs. bottom quartile HR, 1.09; 95% CI, 0.77–1.55; P_{trend} , 0.96) or women (HR, 0.85; 95% CI, 0.61–1.17; P_{trend} , 0.39; Table 2). Results remained null when meta-analyzed (top quartile P_{diff} , 0.30; HR, 0.95; 95% CI, 0.74–1.22; P_{trend} , 0.58). In men, restriction to never-smokers yielded results more suggestive but nonsignificant (HR, 1.59; 95% CI, 0.93–2.72; P_{trend} , 0.09). The meta-analysis of never-smokers showed nonsignificant heterogeneity between the cohorts (top quartile P_{diff} , 0.26), and the combined results were null (HR, 1.27; 95% CI, 0.85–1.91; P_{trend} , 0.13). Results for fatal RCC were similarly suggestive but nonsignificant in men (HR, 1.82; 95% CI, 0.94–3.52; P_{trend} , 0.13) and null in the cohorts combined (top quartile P_{diff} , 0.26; HR, 1.38; 95% CI, 0.84–2.28; P_{trend} , 0.14). There was no association between dietary acrylamide and clear cell RCC. Baseline acrylamide intake and cumulative average intakes of high-acrylamide food

groups, breads, baked goods, cereal, potatoes, and coffee, were not significantly associated with RCC risk in either cohort or when meta-analyzed.

Discussion

We found no association between dietary acrylamide intake and RCC risk in two cohorts with >2 decades of follow-up. A meta-analysis of five studies with 1,802 cases found an RR of 1.20 (95% CI, 1.00–1.45) for the highest versus lowest categories, with an RR of 1.48 (95% CI, 1.09–2.00) for the two prospective cohort studies (2). The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study found no significant association based on 184 cases and higher acrylamide intakes than in our cohorts, driven mainly by coffee (7). The Netherlands Cohort Study found a significant association based on 339 cases and a similar distribution of acrylamide intake to our cohorts (8). However, the major source of variation in acrylamide intake was Dutch spiced cake, which is an uncommon source of acrylamide in other populations. With 629 RCC cases, our cohorts had more cases than the previous prospective studies combined (523 cases). Our study also benefited from repeated measures of diet, which better estimate long-term intakes and reduce random within-person measurement error (5).

Table 1. Age-adjusted characteristics of the study population at baseline (1980 for the NHS and 1986 for the HPFS) according to quartiles of energy-adjusted dietary acrylamide intake

Characteristic	Baseline dietary acrylamide intake quartile ($\mu\text{g/day}$)							
	Men (HPFS)				Women (NHS)			
	Quartile 1 Range: ≤ 14.64 Median: 11.06	Quartile 2 14.65–20.16 17.42	Quartile 3 20.17–27.46 23.23	Quartile 4 ≥ 27.47 35.11	Quartile 1 ≤ 10.19 7.12	Quartile 2 10.20–15.05 12.65	Quartile 3 15.06–20.80 17.63	Quartile 4 ≥ 20.81 25.80
Number	12,484	12,027	11,774	11,512	22,593	22,147	22,041	21,986
Mean age, years (SD) ^a	57.1 (9.7)	55.2 (9.7)	53.8 (9.5)	51.5 (9.2)	48.3 (7.1)	47.2 (7.2)	46.4 (7.1)	45.1 (6.9)
Mean BMI, kg/m^2 (SD)	25.4 (3.5)	25.5 (3.3)	25.5 (3.2)	25.7 (3.4)	24.6 (4.7)	24.4 (4.6)	24.3 (4.4)	24.3 (4.4)
Diagnosis of hypertension	24.4%	21.8%	20.8%	20.8%	19.7%	17.0%	14.8%	13.4%
Diabetes	3.1%	3.1%	3.3%	3.1%	3.1%	2.3%	2.1%	1.7%
Smoking status								
Never	51.9%	48.7%	44.6%	41.7%	49.1%	47.2%	42.6%	35.6%
Past, quit >10 years before baseline	29.0%	30.2%	31.7%	30.5%	17.0%	17.1%	16.3%	13.8%
Past, quit ≤ 10 years before baseline	11.2%	12.0%	13.7%	14.7%	11.7%	10.9%	11.5%	11.1%
Current	7.9%	9.1%	10.1%	13.2%	22.2%	24.8%	29.6%	39.5%
Mean pack-years of smoking (SD) ^b	23.9 (19.0)	24.9 (19.0)	25.5 (19.2)	27.9 (20.1)	19.0 (16.5)	19.2 (16.4)	20.5 (16.6)	23.0 (17.0)
Nulliparous	—	—	—	—	6.3%	5.9%	5.5%	5.7%
Mean parity, number of children (SD) ^c	—	—	—	—	3.1 (1.5)	3.2 (1.5)	3.2 (1.5)	3.2 (1.5)
Mean (SD) nutrient & food intakes								
Total calories, kcal/day	1,997 (641)	2,061 (615)	1,931 (569)	1,943 (640)	1,569 (509)	1,615 (504)	1,576 (473)	1,499 (513)
Alcohol, g/day	11.8 (17.2)	12.1 (16.0)	11.1 (14.2)	10.0 (13.6)	6.5 (11.4)	6.6 (11.0)	6.4 (10.1)	5.7 (9.3)
Breads, servings/day ^d	1.6 (1.3)	2.0 (1.5)	2.1 (1.6)	2.1 (1.6)	1.3 (1.0)	1.5 (1.1)	1.7 (1.2)	1.7 (1.3)
Baked goods, servings/week ^e	4.9 (6.6)	6.4 (7.8)	6.3 (7.5)	6.4 (7.5)	2.5 (3.2)	3.8 (4.8)	4.6 (6.1)	5.5 (7.4)
Cereal, servings/week	2.1 (2.5)	2.7 (2.8)	3.1 (3.1)	3.4 (4.6)	1.7 (2.4)	2.1 (2.6)	2.1 (2.8)	2.1 (3.2)
Potatoes, servings/week ^f	2.7 (2.4)	3.4 (2.4)	3.8 (2.5)	5.5 (3.6)	2.3 (2.2)	3.0 (2.4)	3.4 (2.5)	4.8 (3.8)
Coffee, cups/day ^g	1.0 (1.2)	1.7 (1.5)	2.3 (1.8)	2.8 (2.1)	0.8 (1.0)	1.8 (1.6)	2.7 (1.8)	3.6 (1.9)

NOTE: Percentages may not add up as expected due to rounding.

Abbreviation: BMI, body mass index.

^aNot adjusted for age.

^bAmong 24,396 male ever smokers and 49,822 female ever smokers.

^cAmong 82,158 parous women.

^dBreads include white bread, rye bread, other dark bread, English muffins/bagels/rolls, muffins/biscuits, pancakes/waffles, crackers, pizza, tortillas, pretzels, breakfast bars, energy bars, and high-protein bars.

^eBaked goods include cookies, cake, pie, brownies, doughnuts, and sweet rolls/coffee cake/other pastries.

^fPotatoes include baked/boiled/mashed potatoes, French fries, and potato chips.

^gCoffee includes regular, decaffeinated, and dairy coffee drinks.

Table 2. HR and 95% CI for quartiles of energy-adjusted dietary acrylamide intake and risk of total RCC in the full cohorts and among never-smokers, and risk of fatal RCC and clear cell RCC in the full cohorts

	Men (HPFS)			Women (NHS)		
	# Cases	Simple HR (95% CI) ^a	Multivariable HR (95% CI) ^b	# Cases	Simple HR (95% CI) ^a	Multivariable HR (95% CI) ^b
Total RCC						
Full cohort						
Quartile 1	66	1.00 (ref.)	1.00 (ref.)	82	1.00 (ref.)	1.00 (ref.)
Quartile 2	86	1.32 (0.95–1.82)	1.32 (0.95–1.84)	87	0.94 (0.69–1.27)	0.93 (0.69–1.27)
Quartile 3	68	1.04 (0.73–1.46)	1.01 (0.71–1.43)	96	1.02 (0.76–1.38)	1.01 (0.75–1.36)
Quartile 4	72	1.21 (0.86–1.70)	1.09 (0.77–1.55)	72	0.87 (0.63–1.19)	0.85 (0.61–1.17)
<i>P</i> _{trend}		0.54	0.96		0.47	0.39
Never-smokers						
Quartile 1	25	1.00 (ref.)	1.00 (ref.)	39	1.00 (ref.)	1.00 (ref.)
Quartile 2	30	1.16 (0.68–2.00)	1.24 (0.72–2.14)	42	1.01 (0.65–1.57)	1.00 (0.64–1.55)
Quartile 3	33	1.37 (0.80–2.34)	1.42 (0.83–2.44)	41	1.08 (0.69–1.68)	1.07 (0.68–1.67)
Quartile 4	34	1.63 (0.96–2.78)	1.59 (0.93–2.72)	29	1.06 (0.65–1.73)	1.05 (0.64–1.71)
<i>P</i> _{trend}		0.06	0.09		0.76	0.79
Fatal RCC						
Full cohort						
Quartile 1	16	1.00 (ref.)	1.00 (ref.)	24	1.00 (ref.)	1.00 (ref.)
Quartile 2	24	1.68 (0.89–3.19)	1.71 (0.90–3.25)	19	0.81 (0.44–1.49)	0.79 (0.43–1.45)
Quartile 3	20	1.51 (0.77–2.94)	1.48 (0.76–2.90)	21	0.93 (0.51–1.68)	0.89 (0.49–1.61)
Quartile 4	24	2.02 (1.06–3.85)	1.82 (0.94–3.52)	23	1.13 (0.63–2.02)	1.09 (0.60–1.97)
<i>P</i> _{trend}		0.05	0.13		0.59	0.68
Clear cell RCC						
Full cohort						
Quartile 1	38	1.00 (ref.)	1.00 (ref.)	58	1.00 (ref.)	1.00 (ref.)
Quartile 2	53	1.36 (0.89–2.07)	1.34 (0.87–2.04)	61	0.91 (0.64–1.31)	0.92 (0.64–1.33)
Quartile 3	42	1.04 (0.67–1.62)	0.98 (0.62–1.53)	59	0.88 (0.61–1.27)	0.88 (0.61–1.27)
Quartile 4	46	1.25 (0.81–1.94)	1.09 (0.70–1.70)	54	0.92 (0.63–1.34)	0.92 (0.63–1.35)
<i>P</i> _{trend}		0.56	0.91		0.66	0.67

^aStratified by age in months and calendar time.^bAdditionally adjusted for BMI (<23, 23–<25, 25–<27, and ≥27 kg/m²), history of hypertension (yes and no), history of diabetes (yes and no), smoking status (analyses including smokers only; never, former/quit >10 years ago, former/quit ≤10 years ago, and current), pack-years (analyses including smokers only; continuous), duration of nonaspirin nonsteroidal anti-inflammatory drug use (<5 years and ≥5 years), energy intake (continuous), alcohol consumption (quartiles), and parity (NHS only; 0, 1–2, 3, and ≥4 children).

In conclusion, our results suggest there is not an important association between acrylamide intake and RCC risk, adding to the body of evidence that dietary acrylamide is not an important risk factor for cancer in humans.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The authors assume full responsibility for analyses and interpretation of these data.

Authors' Contributions

Conception and design: R.E. Graff, E. Cho, L.A. Mucci, K.M. Wilson

Development of methodology: R.E. Graff, E. Cho

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E. Cho, M.A. Preston

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R.E. Graff, E. Cho, M.A. Preston, A. Sanchez, K.M. Wilson

Writing, review, and/or revision of the manuscript: R.E. Graff, E. Cho, M.A. Preston, A. Sanchez, L.A. Mucci, K.M. Wilson

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References

- International Agency for Research on Cancer. Acrylamide. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Industrial Chemicals. United Kingdom. Lyon, France: International Agency for Research on Cancer; 1994. p. 389–433.
- Pelucchi C, Bosetti C, Galeone C, La Vecchia C. Dietary acrylamide and cancer risk: an updated meta-analysis. *Int J Cancer* 2015;136:2912–22.
- Cho E, Curhan G, Hankinson SE, Kantoff P, Atkins MB, Stampfer M, et al. Prospective evaluation of analgesic use and risk of renal cell cancer. *Arch Intern Med* 2011;171:1487–93.
- Wilson KM, Vesper HW, Tocco P, Sampson L, Rosen J, Hellenas KE, et al. Validation of a food frequency questionnaire measurement of dietary acrylamide intake using hemoglobin adducts of acrylamide and glycidamide. *Cancer Causes Control* 2009;20:269–78.

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5. Hu FB, Stampfer MJ, Rimm E, Ascherio A, Rosner BA, Spiegelman D, et al. Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. *Am J Epidemiol* 1999;149:531–40.
6. Lopez-Beltran A, Scarpelli M, Montironi R, Kirkali Z. 2004 WHO classification of the renal tumors of the adults. *Eur Urol* 2006;49:798–805.
7. Hirvonen T, Kontto J, Jestoi M, Valsta L, Peltonen K, Pietinen P, et al. Dietary acrylamide intake and the risk of cancer among Finnish male smokers. *Cancer Causes Control* 2010;21:2223–9.
8. Hogervorst JC, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. Dietary acrylamide intake and the risk of renal cell, bladder, and prostate cancer. *Am J Clin Nutr* 2008;87:1428–38.

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Dietary acrylamide intake and the risk of colorectal cancer with specific mutations in KRAS and APC

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Acrylamide, a probable human carcinogen, is present in heat-treated carbohydrate-rich foods. Epidemiological studies have not shown a clear association between acrylamide intake and colorectal cancer (CRC) risk. This may be due to the molecular heterogeneity in colorectal tumors, which was not taken into consideration before. Since the acrylamide metabolite glycidamide induces specific DNA mutations in rodents, we investigated whether acrylamide is associated with CRC risk characterized by mutations in Kirsten-ras (*KRAS*) and adenomatous polyposis coli (*APC*); key genes in colorectal carcinogenesis. This case-cohort analysis, within the Netherlands Cohort Study on diet and cancer, was based on 7.3 years of follow-up. Acrylamide intake was assessed with a food frequency questionnaire. Mutation analysis of codons 1286–1520 in exon 15 in *APC* and codons 12 and 13 in exon 1 in *KRAS* was performed on tumor tissue of 733 cases. Hazard ratios (HR) were calculated using Cox proportional hazards analysis. Among men, acrylamide intake was statistically significantly associated with an increased risk of particularly tumors with an activating *KRAS* mutation (HR fourth versus first quartile: 2.12 [95% confidence interval (CI): 1.16–3.87], *P* trend: 0.01). Among women, acrylamide intake was statistically significantly associated with a decreased risk of particularly tumors with a truncating *APC* mutation (fourth versus first quartile: 0.47 (95% CI: 0.23–0.94), *P* trend: 0.02), but only in the highest quartile of intake. This is the first study to show that acrylamide might be associated with CRC with specific somatic mutations, differentially in men and women. More research is needed to corroborate or refute these findings.

Introduction

In Europe, in 2008, colorectal cancer (CRC) was the most common cancer (436 000 cases; 13.6% of all cancer cases) and the second leading cause of cancer death (212 000 deaths; 12.3%) (1). Throughout the world, CRC incidence rates vary widely, which is likely to be the result of environmental factors, especially specific components in the diet.

Acrylamide is used in industrial chemistry as a precursor in the production of polyacrylamides, which are used for clarifying drinking water and other industrial applications. Acrylamide is a neurotoxin in humans and has shown to be carcinogenic in rodents (2). In 1994, it was classified by the International Agency for Research on Cancer as a probable human carcinogen (group 2A) (2). In 2002, the Swedish

National Food Administration reported high levels of acrylamide in commonly consumed heat-processed foods (3), such as French fries, potato chips and cookies.

In rodents, acrylamide given in drinking water led to several tumors, especially in hormone-sensitive organs such as the testis and the mammary gland (4,5). *In vivo*, acrylamide is oxidized to the epoxide glycidamide, catalyzed by the enzyme cytochrome P4502E1 (CYP2E1) (6). Contrary to acrylamide itself, glycidamide forms adducts with DNA bases and is mutagenic (7). In rodents, glycidamide shows a characteristic DNA mutation pattern. The most frequently observed mutations induced by acrylamide administration were A>G transitions and G>C transversions. Direct administration of glycidamide appeared to induce more mutations than acrylamide at any given dose and additionally rendered G>T transversions (7–9).

A hormonal pathway for acrylamide-induced carcinogenesis has been hypothesized (10). In rats, acrylamide exposure has been shown to influence hormone levels (11–13), and in mice (14,15) and human breast and colorectal cells, acrylamide increased the expression of genes involved in the generation of sex hormones (16).

In epidemiological studies, no clear association was found between acrylamide exposure and CRC risk. One case-control study observed a statistically significant inverse association, hypothesized to be due to residual confounding (17). Three prospective cohort studies [among which was the Netherlands Cohort Study on diet and cancer (NLCS) study using 13.3 years of follow-up] and a case-control study did not show an association between acrylamide intake and CRC risk (18–21). This may either indicate that acrylamide has no role in causing CRC or the studies may have missed a true association. Associations may become more apparent when the molecular heterogeneity of colorectal tumors is taken into account.

The inactivation of the tumor suppressor gene adenomatous polyposis coli (*APC*) and the activation of the proto-oncogene Kirsten-ras (*KRAS*) are thought to be key events in CRC initiation and progression (22). *APC* has been proposed to function as a ‘gatekeeper’ gene, and inactivation of the *APC* gene seems to trigger the cascade of events that leads to malignant transformation of epithelial cells into adenocarcinoma (23). Somatic mutations in the *APC* gene are found in the majority of sporadic colorectal tumors (24) and most of these mutations occur within the codons 1286–1520 of exon 15, the so-called mutation cluster region (25). Missense or frameshift mutations in the *APC* gene lead to truncated, and therefore inactive *APC* proteins (22). Mutations in *KRAS* are thought to lead to increased and unregulated cellular proliferation and malignant transformation from an intermediate adenoma to a late adenoma or carcinoma. About 30–60% of the colorectal adenocarcinomas have a *KRAS* mutation (26). The most frequently affected codons are codons 12 and 13 in exon 1 and to a lesser extent codon 61 in exon 2.

We hypothesized that acrylamide intake is associated with the risk of CRC with a specific molecular signature characterized by activating mutations in the *KRAS* gene and truncating mutations in the *APC* gene and we focused on the specific point mutations induced by acrylamide and its metabolite glycidamide in rodents: G>C transversions, G>T transversions and A>G transitions. The latter mutation can, however, not be investigated in the current study, because from a previous analysis, it was known that A>G mutations do not lead to truncating *APC* mutations in this study population (27) and activating *KRAS* mutations are only G mutations.

Materials and methods

Study design

This study was embedded in the NLCS. The NLCS was initiated in September 1986 and included 58 279 men and 62 573 women aged 55–69 years, who

Abbreviations: AA, acrylamide-associated; APC, adenomatous polyposis coli; CI, confidence interval; CRC, colorectal cancer; FFQ, food frequency questionnaire; HR, hazard ratio; KRAS, Kirsten-ras; NLCS, Netherlands Cohort Study on diet and cancer.

were identified through 204 municipal computerized population registries throughout the Netherlands (28). The study design for the present analyses was a case-cohort study: cases were accumulated from the entire NLCS cohort and a subcohort ($n = 5000$) was randomly selected from the entire cohort at baseline. The number of person-years at risk for the entire cohort was estimated from the subcohort, for efficiency reasons.

Identification of the incident histologically confirmed CRC cases was done by record linkage to the Netherlands Cancer Registry and the Dutch Pathology Registry (in Dutch: PALGA), providing a near 100% coverage of the municipalities included in the NLCS (29). Cases and subcohort members were excluded from the analyses if they had been diagnosed with another cancer (except skin cancer) at baseline and if their dietary data were incomplete or inconsistent. Due to incomplete coverage by PALGA in the earlier years, the first 2.3 years of follow-up were excluded from the analyses.

Tissue samples

As described previously, tumor material of CRC cases was collected after approval by the ethical review boards of Maastricht University, the Netherlands Cancer Registry and PALGA (30). All relevant pathology laboratories in the Netherlands agreed to make tissue samples available upon request from PALGA. From the 815 eligible tissue samples distributed among 54 pathology laboratories throughout the Netherlands, 771 samples could be retrieved. After excluding samples that contained only healthy colorectal mucosa, that were revised as a benign adenoma instead of a carcinoma by a pathologist or that did not yield sufficient DNA, tumor tissue from 733 CRC patients was available for mutation analysis.

Mutation analysis

The methods of DNA isolation, PCR and sequencing are extensively described elsewhere (27,30). Tumor material was analyzed for mutations in codons 1286–1520 (mutation cluster region) of exon 15 of the *APC* gene and in codons 12 and 13 of exon 1 of the *KRAS* gene using nested PCR, followed by direct sequencing of purified segments. Mutation analysis of the *APC* gene was successful and complete for 662 samples, and of the *KRAS* gene for all 733 samples.

Acrylamide intake assessment

At the start of the NLCS in 1986, the cohort members completed a semiquantitative, self-administered questionnaire on diet, other environmental risk factors, medical history and family history of cancer. The dietary part was a 150 item food frequency questionnaire (FFQ), which assessed habitual consumption (frequency and portion size) of foods and beverages during the year preceding the

start of the study. Acrylamide intake was assessed with this FFQ, as described previously (31). In short, data from the Dutch Food and Consumer Product Safety Authority were used to assign a mean acrylamide concentration to each food from the NLCS FFQ. An estimation of the total dietary acrylamide intake was made using the mean acrylamide level in the foods, and the consumption frequency and portion size of the foods, with a subsequent summation across all of the foods.

Data analysis

Data analysis was conducted for men and women separately, because of the potential sex hormonal effect of acrylamide (10). Tumor DNA contains thousands of mutations, but most mutations do not alter protein function and therefore are unlikely to have played a role in cancer causation. Because of that, we only examined mutations leading to the activation of the *KRAS* gene and to introduction of a stop codon (truncating mutation) in the *APC* gene, which are mutations that are likely to have played an important role in the carcinogenic process. The molecular endpoints that we analyzed were: an activating *KRAS* and/or truncating *APC* mutation; a G>C or G>T activating *KRAS* and/or truncating *APC* mutation; an activating *KRAS* mutation; a G>C or G>T activating *KRAS* mutation; a truncating *APC* mutation and a truncating G>C or G>T *APC* mutation. In addition, we zoomed in on the singular mutations leading to activating *KRAS* and truncating *APC* mutations. From now on, for brevity, we will talk about *KRAS* mutations when we mean activating *KRAS* mutations and about *APC* mutations when we mean truncating *APC* mutations. For readability, we will from now on use the wording AA (acrylamide-associated) mutations to signify G>C or G>T mutations.

Hazard ratios (HR) and corresponding 95% confidence intervals (CI) were determined using Cox proportional hazards models with person-years at risk as the time metric. In subgroup analyses, we restricted to never smokers because of the high concentration of acrylamide in tobacco smoke that may blur the estimation of dietary acrylamide intake. However, for men, the number of never smokers was too small, and therefore, we combined never smokers with ex-smokers that quit >10 years before baseline. In order to avoid analyses in very small groups, acrylamide intake was included in the models as a continuous variable, as tertiles or as quartiles with a minimum of 20, 60 and 80 cases, respectively. When there were <60 cases, acrylamide intake was only modeled continuously. HRs were adjusted for age and variables known as CRC risk factors in this population (*a priori* chosen covariables), namely smoking (status, quantity and duration), body mass index, family history of CRC and total energy intake. Variables considered as potential confounders were included in the multivariable-adjusted model if they changed the age-adjusted HR of acrylamide by >10%. The following variables, selected from the literature, were tested: physical activity, education level, intake of fat, fiber,

Table I. Characteristics of male CRC cases with specific molecular endpoints and male subcohort members^a

	Subcohort	Case groups						
		Total CRC	Activating <i>KRAS</i> and/or truncating <i>APC</i> mutation	G>C or G>T activating <i>KRAS</i> and/or truncating <i>APC</i> mutation	Activating <i>KRAS</i> mutation	G>C or G>T activating <i>KRAS</i> mutation	Truncating <i>APC</i> mutation	G>C or G>T truncating <i>APC</i> mutation
<i>n</i> ^b	1904	341	183	72	114	48	117	28
Acrylamide intake, µg/day	22	23	24	25	24	25	23	26
Acrylamide intake, µg × kg·BW ⁻¹ × day ⁻¹	0.29	0.29	0.31	0.32	0.31	0.32	0.30	0.34
Main food sources of acrylamide								
Coffee, g/day	573	560	591	598	586	581	603	621
Dutch spiced cake, g/day	4	5	5	6	5	6	4	8
Cookies, g/day	14	14	14	16	15	18	13	14
Potato crisps, g/day	0.43	0.55	0.53	0.48	0.60	0.37	0.42	0.69
French fries, g/day	7	7	8	6	8	6	8	6
<i>A priori</i> chosen covariables								
Age, years	61	63	63	63	63	63	63	61
BMI, kg/m ²	25	25	26	25	26	25	25	25
Family history of CRC, <i>n</i> (% yes)	6	12	11	14	11	13	12	14
Total energy intake, kJ/day	2173	2139	2162	2193	2139	2146	2166	2258
Smoking status, %								
Never	14	11	10	10	11	13	10	4
Ex-smoker	52	64	64	64	64	62	62	64
Current smoker	34	25	26	26	25	25	28	32
Duration of cigarette smoking, years	29	30	30	32	30	31	31	34
Frequency of cigarette smoking, <i>n</i> /day	15	17	17	15	18	14	16	16

BMI, body mass index; BW, body weight.

^aData represent means or percentages.

^b*n* without missing values for *a priori* chosen covariables.

Table II. HR and 95% CI for CRC risk according to categories of dietary acrylamide intake among men

Endpoint		Continuous acrylamide intake (per 10 µg/day)	Quartile 1/tertile 1	Quartile 2/tertile 2	Quartile 3/tertile 3	Quartile 4	P trend
Total CRC							
Smokers and non-smokers	<i>n</i> cases/ <i>n</i> person-years	341/9115	86/2246	85/2281	76/2335	94/2253	
	HR (95% CI) ^a	1.03 (0.94–1.14)	1.00 (ref)	1.10 (0.78–1.55)	1.00 (0.70–1.43)	1.17 (0.82–1.66)	0.44
Non-smokers ^b	<i>n</i> cases/ <i>n</i> person-years	255/7285	60/1761	69/1836	55/1853	71/1835	
	HR (95% CI) ^a	1.02 (0.92–1.14)	1.00 (ref)	1.31 (0.88–1.96)	1.10 (0.73–1.66)	1.32 (0.88–1.99)	0.32
KRAS and/or APC mutation							
Smokers and non-smokers	<i>n</i> cases/ <i>n</i> person-years	183/9115	39/2246	39/2281	47/2335	58/2253	
	HR (95% CI) ^a	1.10 (0.98–1.23)	1.00 (ref)	1.11 (0.69–1.79)	1.36 (0.85–2.18)	1.58 (1.00–2.51)	0.04
Non-smokers ^b	<i>n</i> cases/ <i>n</i> person-years	140/7285	27/1761	34/1836	35/1853	44/1835	
	HR (95% CI) ^a	1.07 (0.94–1.21)	1.00 (ref)	1.37 (0.79–2.37)	1.49 (0.86–2.57)	1.72 (1.00–2.95)	0.07
AA KRAS and/or APC mutation							
Smokers and non-smokers	<i>n</i> cases/ <i>n</i> person-years	72/9115	19/3038	23/3088	30/2989		
	HR (95% CI) ^a	1.13 (0.96–1.34)	1.00 (ref)	1.29 (0.70–2.41)	1.66 (0.88–3.11)		0.12
Non-smokers ^b	<i>n</i> cases/ <i>n</i> person-years	55/7285	15/2390	17/2433	23/2462		
	HR (95% CI) ^a	1.09 (0.91–1.31)	c	c	c	c	c
KRAS mutation							
Smokers and non-smokers	<i>n</i> cases/ <i>n</i> person-years	114/9115	21/2246	24/2281	30/2335	39/2253	
	HR (95% CI) ^a	1.15 (1.00–1.31)	1.00 (ref)	1.34 (0.72–2.49)	1.76 (0.96–3.22)	2.12 (1.16–3.87)	0.01
Non-smokers ^b	<i>n</i> cases/ <i>n</i> person-years	87/7285	13/1761	21/1836	21/1853	32/1835	
	HR (95% CI) ^a	1.16 (1.00–1.33)	1.00 (ref)	1.84 (0.89–3.80)	1.99 (0.96–4.13)	2.78 (1.37–5.67)	0.007
AA KRAS mutation							
Smokers and non-smokers	<i>n</i> cases/ <i>n</i> person-years	48/9115	11/3038	16/3088	21/2989		
	HR (95% CI) ^a	1.17 (0.96–1.42)	c	c	c	c	c
Non-smokers ^b	<i>n</i> cases/ <i>n</i> person-years	37/7285	9/2390	12/2433	16/2462		
	HR (95% CI) ^a	1.16 (0.94–1.43)	c	c	c	c	c
APC mutation							
Smokers and non-smokers	<i>n</i> cases/ <i>n</i> person-years	117/9115	28/2246	25/2281	32/2335	32/2253	
	HR (95% CI) ^a	1.04 (0.91–1.20)	1.00 (ref)	0.94 (0.53–1.66)	1.22 (0.71–2.11)	1.16 (0.67–2.02)	0.49
Non-smokers ^b	<i>n</i> cases/ <i>n</i> person-years	91/7285	20/1761	22/1836	27/1853	22/1835	
	HR (95% CI) ^a	0.99 (0.84–1.15)	1.00 (ref)	1.13 (0.59–2.17)	1.47 (0.79–2.71)	1.09 (0.56–2.11)	0.84
AA APC mutation							
Smokers and non-smokers	<i>n</i> cases/ <i>n</i> person-years	28/9115	8/3038	7/3088	13/2989		
	HR (95% CI) ^a	1.15 (0.91–1.46)	c	c	c	c	c
Non-smokers ^b	<i>n</i> cases/ <i>n</i> person-years	20/7285	6/2390	5/2433	10/2462		
	HR (95% CI) ^a	1.01 (0.77–1.32)	c	c	c	c	c

Median acrylamide intake in the quartiles of acrylamide intake in men (subcohort): 11.7, 17.0, 23.0 and 35.8 µg/day.

^aAdjusted for age (years), smoking [status (current/non-current), quantity (*n* cigarettes/day) and duration (years)], body mass index (kg/m²), family history of CRC (yes/no) and total energy intake (kcal/day).

^bNon-smokers: never smokers and ex-smokers that quit smoking >10 years before baseline.

^cInsufficient number of cases.

Table III. Endpoints for which the scaled Schoenfeld residuals indicated violation of the proportional hazards assumption: analysis stratified by follow-up time

Endpoint	2.3–4.8 years of follow-up		4.8–7.3 years of follow-up	
	<i>n</i> cases/ <i>n</i> person-years	HR (95% CI)	<i>n</i> cases/ <i>n</i> person-years	HR (95% CI)
KRAS and/or APC mutation, men ^a	76/4667	1.23 (1.06–1.42)	107/4448	1.00 (0.86–1.16)
AA KRAS and/or APC mutation, men	33/4667	1.33 (1.08–1.62)	39/4448	0.95 (0.73–1.24)
KRAS mutation, men ^b	45/4667	1.30 (1.07–1.57)	69/4448	1.06 (0.89–1.25)
APC mutation, women	46/5162	0.69 (0.47–1.01)	40/5033	1.10 (0.87–1.38)
APC mutation, never smokers, women	31/3087	0.66 (0.39–1.11)	28/3024	1.13 (0.82–1.56)

HRs adjusted for age (years), smoking [status (current/non-current), quantity (*n* cigarettes/day) and duration (years)], body mass index (kg/m²), family history of CRC (yes/no) and total energy intake (kcal/day).

^aKRAS and/or APC mutation, men: 2.3–4.8 years of follow-up: tertile 2: 1.61 (0.85–3.03), tertile 3: 2.35 (1.26–4.37), *P* = 0.01; 4.8–7.3 years of follow-up: tertile 2: 1.17 (0.71–1.95), tertile 3: 1.07 (0.64–1.81), *P* = 0.88.

^bKRAS mutation, men: 4.8–7.3 years of follow-up: tertile 2: 1.38 (0.73–2.63), tertile 3: 1.37 (0.71–2.64), *P* = 0.43.

heme iron, dietary vitamin B6, vegetables, fruits, dairy, meat, alcohol and tea. In addition, carbohydrate and *trans* unsaturated fatty acid intake were checked because of their correlation with acrylamide intake. Dose–response trends were tested by fitting the median acrylamide intake per quantile as a continuous variable and evaluated using the Wald χ^2 test.

The proportional hazards assumption was checked using scaled Schoenfeld residuals and by visually inspecting the $-\ln[-\ln(\text{survival})]$ curves. Standard errors were estimated using the robust Huber–White sandwich estimator to account for additional variance introduced by sampling from the cohort.

All statistical analyses were performed using STATA version 12 and the tests were performed two sided with a *P* value <0.05 considered as statistically significant.

Results

For the Cox proportional hazards analyses, only the *a priori* chosen covariables: smoking (status, quantity and duration), body mass index,

family history of CRC and total energy intake were included in the multivariable-adjusted models, since adding the potential confounders did not change the HRs by >10%. Multivariable-adjusted results did not differ importantly from age-adjusted results and did not lead to different conclusions. Therefore, only the multivariable-adjusted results are shown and discussed; first for men and then for women.

Men

At baseline, male cases were older than male subcohort members (except cases with a tumor with an AA *APC* mutation) and more often showed a family history of CRC (Table I).

Among men, acrylamide intake was not associated with CRC risk overall (Table II). There was a statistically significant positive, dose-dependent association between acrylamide intake and tumors with a *KRAS* and/or *APC* mutation (HR for the highest versus the lowest quartile = 1.58 (95% CI: 1.00–2.51), *P* trend: 0.04), and the HR was 1.10 (95% CI: 0.98–1.23) per 10 µg/day increment. Among non-smoking men, the corresponding HRs were 1.72 (95% CI: 1.00–2.95; *P* trend: 0.07) and 1.07 (95% CI: 0.94–1.21), respectively. When looking at tumors with an AA *KRAS* and/or *APC* mutation, weaker associations were seen when looking at the tertiles of acrylamide intake: HR for the highest versus the lowest tertile = 1.66 (95% CI: 0.88–3.11; *P* trend: 0.12), but the HR per 10 µg/day increment was 1.13 (95% CI: 0.96–1.34). Among non-smoking men, the HR per 10 µg/day increment was 1.09 (95% CI: 0.91–1.31). The number of non-smokers was too small for an analysis based on categories of acrylamide intake for this endpoint. It appeared that the abovementioned associations were driven by the association with the risk of tumors with a *KRAS* mutation, because the HR for tumors with a *KRAS* mutation were stronger: the HR for the highest versus the lowest quartile was 2.12 (95% CI: 1.16–3.87; *P* trend: 0.01) and a HR of 1.15 (95% CI: 1.00–1.31) per 10 µg/day increment. Among non-smokers, the corresponding HRs were 2.78 (95% CI: 1.37–5.67; *P* trend: 0.007) and 1.16 (95% CI: 1.00–1.33). There were not enough cases to look at the dose–response relationship, but the HR per 10 µg/day increment for a tumor with an AA *KRAS* mutation was slightly higher than for a tumor with any kind of activating *KRAS* mutation: 1.17 (95% CI: 0.96–1.42) for all men and 1.16 (95% CI: 0.94–1.43) for non-smokers. Acrylamide intake was not statistically significantly associated with tumors with an *APC* mutation among men, but the continuous acrylamide variable was associated with the risk of tumors with an AA *APC* mutation with approximately the same strength as with the risk of a tumor with an AA *KRAS* mutation. The small number of cases precluded looking at the dose–response relationship for this endpoint. Among non-smoking men, this association was considerably reduced.

The proportional hazards assumption was violated according to the scaled Schoenfeld residuals for the continuous acrylamide intake and the highest category of acrylamide intake in the analyses of tumors with a *KRAS* and/or *APC* mutation, tumors with an AA *KRAS* and/or *APC* mutation and tumors with a *KRAS* mutation. However, only for tumors with a *KRAS* and/or *APC* mutation there was a borderline statistically significant interaction with follow-up time. Inspection of the $-\ln[-\ln(\text{survival})]$ curves showed crossing of survival curves for the different acrylamide intake categories. The results of splitting of the follow-up period halfway are presented in Table III. The positive associations appeared to be confined to the follow-up period 2.3–4.8 years for tumors with a *KRAS* and/or *APC* mutation, tumors with an AA *KRAS* and/or *APC* mutation and tumors with a *KRAS* mutation.

Table IV shows the associations between acrylamide intake and the risk of CRC stratified by the singular point mutations. Among men, a borderline statistically significant positive association was observed between acrylamide intake and the risk of tumors with an G:C>T:A activating *KRAS* mutation [HR per 10 µg/day: 1.22 (95% CI: 0.99–1.50)]. For G:C>A:T activating *KRAS* mutations, there was a statistically non-significant positive association with acrylamide intake, whereas the number of G:C>C:G activating *KRAS* mutations was too small for a meaningful analysis. Sample sizes were too small for analyses of the singular mutations among non-smoking men.

Table IV. HR and 95% CI for CRC risk specified by specific mutations in the *KRAS* and *APC* gene according to categories of dietary acrylamide intake

	Mutation induced by glycidamide in rodents						Other functional mutation			
	G>T activating <i>KRAS</i> mutation	G>C activating <i>KRAS</i> mutation	G>T truncating <i>APC</i> mutation	G>C truncating <i>APC</i> mutation	A>G truncating <i>APC</i> mutation		G>A activating <i>KRAS</i> mutation ^a	G>A truncating <i>APC</i> mutation ^b	A>T truncating <i>APC</i> mutation	A>C truncating <i>APC</i> mutation
Men										
<i>n</i> cases/person–years	40/13 490	9/13 490	23/13 490	6/13 490	0/13 490		67/13 490	71/13 490	4/13 490	1/13 490
HR (per 10 µg/day increment) (95% CI)	1.22 (0.99–1.50)	0.98 (0.73–1.33)	0.98 (0.73–1.33)	0.98 (0.73–1.33)	0.98 (0.73–1.33)		1.15 (0.97–1.35)	1.11 (0.93–1.31)	1.10 (0.98–1.23)	1.58 (1.00–2.51)
Women										
<i>n</i> cases/person–years	36/14 982	9/14 982	13/14 982	3/14 982	0/14 982		51/14 982	52/14 982	6/14 982	3/14 982
HR (per 10 µg/day increment) (95% CI)	0.79 (0.55–1.14)	1.19 (0.85–1.68)	1.19 (0.85–1.68)	1.19 (0.85–1.68)	1.19 (0.85–1.68)		0.95 (0.72–1.26)	0.82 (0.60–1.11)	1.10 (0.98–1.23)	1.58 (1.00–2.51)

HRs adjusted for age (years), smoking [status (current/non-current), quantity (*n* cigarettes/day) and duration (years)], body mass index (kg/m²), family history of CRC (yes/no) and total energy intake (kcal/day).

^aG:C>A:T activating *KRAS* mutation, men: tertile 2: 1.67 (0.84–3.30), tertile 3: 1.89 (0.98–3.64), *P* = 0.08.

^bG:C>A:T truncating *APC* mutation, men: tertile 2: 1.05 (0.56–1.99), tertile 3: 1.45 (0.80–2.62), *P* = 0.19.

^cInsufficient number of cases.

Table V. Characteristics of female CRC cases with specific molecular endpoints and female subcohort members^a

	Subcohort	Case groups						
		Total CRC	<i>KRAS</i> and/or <i>APC</i> mutation	AA <i>KRAS</i> and/or <i>APC</i> mutation	<i>KRAS</i> mutation	AA <i>KRAS</i> mutation	<i>APC</i> mutation	AA <i>APC</i> mutation
n ^b	2084	282	136	60	94	45	86	16
Acrylamide intake, µg/day	21	20	20	20	20	19	20	23
Acrylamide intake, µg × kg BW ⁻¹ × day ⁻¹	0.32	0.29	0.29	0.27	0.28	0.26	0.29	0.33
Main food sources of acrylamide								
Coffee, g/day	497	518	497	463	490	454	492	503
Dutch spiced cake, g/day	6	5	5	5	5	4	4	7
Cookies, g/day	14	14	14	15	14	15	14	16
Potato crisps, g/day	0.40	0.33	0.38	0.22	0.17	0.24	0.49	0.17
French fries, g/day	4	4	5	4	3	3	6	4
<i>A priori</i> chosen covariables								
Age, years	61	63	63	63	63	63	63	63
BMI, kg/m ²	25	26	26	26	26	26	26	26
Family history of CRC, n (% yes)	6	10	7	5	6	4	8	6
Total energy intake, kJ/day	1684	1668	1723	1737	1707	1728	1737	1733
Smoking status, %								
Never	60	63	65	65	67	67	69	62
Ex-smoker	20	22	20	22	21	20	14	25
Current smoker	20	15	15	13	12	13	17	13
Duration of cigarette smoking, years	11	10	10	10	10	10	9	8
Frequency of cigarette smoking, n/day	5	4	4	3	4	4	3	2

BMI, body mass index; BW, body weight.

^aData represent means (SD) or percentages.^bn without missing values for *a priori* chosen covariables.

Women

Female cases were older at baseline than subcohort members and had a higher body mass index (Table V). In addition, female cases were generally more often never smokers, less often current smokers, and smoked fewer cigarettes per day and had smoked for a shorter duration than subcohort members.

Among women, there was a modest tendency toward an inverse association for all of the endpoints, but this was mostly restricted to the highest acrylamide intake category (Table VI). The most prominently decreased risks were observed for tumors with a *KRAS* and/or *APC* mutation [HR for the highest versus the lowest quartile = 0.59 (95% CI: 0.34–1.02); *P* trend: 0.04], and tumors with an *APC* mutation [HR for the highest versus the lowest quartile = 0.45 (95% CI: 0.23–0.91); *P* trend: 0.02]. Among never-smoking women, the corresponding HRs were 0.42 (95% CI: 0.20–0.88; *P* trend: 0.01) and 0.48 (95% CI: 0.24–0.98; *P* trend: 0.03). Despite the statistically significant *P* for trend in these analyses, the HRs did not decrease consistently over the categories of intake, and the HR per 10 µg/day increment was never statistically significantly <1.

Scaled Schoenfeld residuals indicated a violation of the proportional hazards assumption for the continuous acrylamide variable and the highest category of acrylamide intake for tumors with an *APC* mutation, both among all women and among never-smoking women. Only for the highest quartile of intake for all women was there a statistically significant interaction with follow-up time. Inspection of the $-\ln[-\ln(\text{survival})]$ indicated crossing of the survival curves for the different acrylamide categories. In Table III, it is shown that the inverse association between acrylamide intake and tumors with an *APC* mutation was confined to the follow-up period 2.3–4.8 years.

Only two singular mutations leading to truncation of the *APC* gene could be studied, namely G:C>T:A and G:C>A:T truncating *APC* mutations, and only the former showed a statistically non-significantly increased HR (Table IV). For the other mutations, case numbers were deemed too small. Subgroup analyses among never-smoking women with respect to specific single types of truncating *APC* mutations could not be performed due to the limited case numbers.

Discussion

In this study, we investigated associations between dietary acrylamide intake and the CRC risk characterized by mutations in the *KRAS* and *APC* gene. Since, to the best of our knowledge, this is the first study on acrylamide intake in relation to specific mutations in these genes and we performed many analyses within small subgroups, caution must be exercised in interpreting the results.

Among men, we found a statistically significant positive multivariable-adjusted association between acrylamide intake and the risk of colorectal tumors harboring activating *KRAS* mutations. When we zoomed in to see which specific mutations were behind this, we observed a statistically significant positive association for the only mutation that we were able to study out of the two relevant mutations caused by the acrylamide metabolite glycidamide in rodents, namely G>T mutations.

Among women, a decreased risk in the highest category of acrylamide intake was observed for all molecular endpoints; the most prominent and statistically significant multivariable-adjusted inverse association was found for tumors with a truncating *APC* mutation. There were no indications for any specific singular mutations, but in the case of a putative protective association, this would not be expected. We have no explanation for why acrylamide intake is positively associated with the risk of CRC with activating *KRAS* mutations among men and not among women.

The associations for which a violation of the proportional hazards assumption was shown appeared to be confined to the follow-up period 2.3–4.8 years. We do not have a clear-cut explanation for this, but it calls for a cautious interpretation of the observed associations, especially because we are not able to investigate what the association between acrylamide intake and CRC risk is in the first 2.3 years of follow-up because the first 2.3 years were excluded due to the fact that tumor material of cases was only collected for the subsequent 5 years of follow-up. Protopathic bias, however, is therefore unlikely to have taken place.

The relationship between acrylamide intake and overall CRC risk for men and women separately was only investigated in four other epidemiological studies. A case-control study (20), a cohort study

Table VI. HR and 95% CI for CRC risk according to categories of dietary acrylamide intake among women

Endpoint		Continuous acrylamide intake (per 10 µg/day)	Quartile 1/tertile 1	Quartile 2/tertile 2	Quartile 3/tertile 3	Quartile 4	P trend
Total CRC							
Smokers and non-smokers	<i>n</i> cases/ <i>n</i> person-years	282/10 195	76/2515	74/2568	75/2527	57/2585	
	HR (95% CI) ^a	0.95 (0.85–1.07)	1.00 (ref)	1.00 (0.70–1.43)	1.09 (0.76–1.55)	0.76 (0.52–1.11)	0.13
Never smokers	<i>n</i> cases/ <i>n</i> person-years	179/6111	59/1628	51/1472	47/1467	32/1544	
	HR (95% CI) ^a	0.95 (0.82–1.11)	1.00 (ref)	1.13 (0.73–1.75)	1.10 (0.70–1.74)	0.66 (0.41–1.08)	0.04
KRAS and/or APC mutation							
Smokers and non-smokers	<i>n</i> cases/ <i>n</i> person-years	136/10 195	35/2515	37/2568	41/2527	23/2585	
	HR (95% CI) ^a	0.93 (0.78–1.10)	1.00 (ref)	1.04 (0.64–1.69)	1.17 (0.72–1.91)	0.60 (0.34–1.05)	0.04
Never smokers	<i>n</i> cases/ <i>n</i> person-years	88/6111	24/1628	26/1472	27/1467	11/1544	
	HR (95% CI) ^a	0.87 (0.69–1.11)	1.00 (ref)	1.12 (0.62–2.04)	1.18 (0.64–2.18)	0.42 (0.20–0.88)	0.006
AA KRAS and/or APC mutation							
Smokers and non-smokers	<i>n</i> cases/ <i>n</i> person-years	60/10 195	24/3413	19/3353	17/3429		
	HR (95% CI) ^a	0.86 (0.67–1.11)	1.00 (ref)	0.78 (0.42–1.47)	0.65 (0.33–1.27)		0.24
Never smokers	<i>n</i> cases/ <i>n</i> person-years	39/6111	17/2151	12/1922	10/2038		
	HR (95% CI) ^a	0.82 (0.55–1.20)	^b	^b	^b	^b	^b
KRAS mutation							
Smokers and non-smokers	<i>n</i> cases/ <i>n</i> person-years	94/10 195	24/2515	26/2568	29/2527	15/2585	
	HR (95% CI) ^a	0.90 (0.73–1.10)	1.00 (ref)	1.16 (0.65–2.07)	1.33 (0.75–2.39)	0.61 (0.30–1.20)	0.08
Never smokers	<i>n</i> cases/ <i>n</i> person-years	63/6111	25/2151	23/1922	15/2038		
	HR (95% CI) ^a	0.82 (0.62–1.08)	1.00 (ref)	1.09 (0.58–2.02)	0.63 (0.32–1.28)		0.15
AA KRAS mutation							
Smokers and non-smokers	<i>n</i> cases/ <i>n</i> person-years	45/10 195	18/3413	15/3353	12/3429		
	HR (95% CI) ^a	0.79 (0.58–1.07)	^b	^b	^b	^b	^b
Never smokers	<i>n</i> cases/ <i>n</i> person-years	30/6111	13/2151	9/1922	8/2038		
	HR (95% CI) ^a	0.77 (0.50–1.19)	^b	^b	^b	^b	^b
APC mutation							
Smokers and non-smokers	<i>n</i> cases/ <i>n</i> person-years	86/10 195	24/2515	23/2568	26/2527	13/2585	
	HR (95% CI) ^a	0.90 (0.72–1.12)	1.00 (ref)	0.90 (0.49–1.64)	1.01 (0.56–1.83)	0.47 (0.23–0.94)	0.02
Never smokers	<i>n</i> cases/ <i>n</i> person-years	59/6111	23/2151	22/1922	14/2038		
	HR (95% CI) ^a	0.90 (0.67–1.20)	^b	^b	^b		^b
AA APC mutation							
Smokers and non-smokers	<i>n</i> cases/ <i>n</i> person-years	16/10 195	6/3413	4/3353	6/3429		
	HR (95% CI) ^a	^b	^b	^b	^b	^b	^b
Never smokers	<i>n</i> cases/ <i>n</i> person-years	11/6111	5/2151	3/1922	3/2038		
	HR (95% CI) ^a	^b	^b	^b	^b	^b	^b

Median acrylamide intake in the quartiles of acrylamide intake among women (subcohort): 10.2, 15.2, 21.2 and 35.0 µg/day; tertiles: 11.4, 17.9 and 32.0 µg/day.

^aAdjusted for age (years), smoking [status (current/non-current), quantity (*n* cigarettes/day) and duration (years)], body mass index (kg/m²), family history of CRC (yes/no) and total energy intake (kcal/day).

^bInsufficient number of cases.

among men (18), a cohort study among women (19) and the NLCS study with a follow-up of 13.3 years (21) did not observe a statistically significant association. Mucci *et al.* (17) reported an inverse association among non-smokers (men and women combined), a subgroup that probably contains a substantial amount of women.

Our study has some limitations. Most importantly, chance findings due to multiple subgroup analyses cannot be excluded, although the subgroups were *a priori* chosen. Another limitation is the potential non-differential misclassification of acrylamide exposure assessed with a FFQ. However, a validation study showed that using mean acrylamide levels for individual foods does not preclude reliable ranking of consumers in terms of their acrylamide intake (32). Together with the high validity and reproducibility of the NLCS FFQ (33,34), this strengthens our confidence that the acrylamide intake assessment in our study was of reasonable quality.

Concurrently with acrylamide, people consuming heat-generated foods are exposed to a number of other carcinogenic substances in those foods, e.g. polycyclic aromatic hydrocarbons, furans and furan derivatives, such as 5-hydroxymethylfurfural, and these other compounds may thus be confounders in the relationship between acrylamide intake and cancer risk. However, the reaction in which acrylamide is generated heavily relies on specific conditions, such as the presence of asparagine, whereas for other heat-generated toxicants other specific conditions are needed. Thus, across the span of a whole diet, the correlation between acrylamide and other heat-generated

compounds is probably limited and there seems little potential for confounding. For 5-hydroxymethylfurfural this has been clearly shown, as no clear correlation between the estimated dietary intake of 5-hydroxymethylfurfural and acrylamide was observed in a correlation study (35).

The strengths of this study are the prospective design, the selection of an older cohort, in which dietary habits are relatively stable and the large size of this cohort. The combination of available molecular data and detailed information on several potential confounding factors provided by a validated and reproducible FFQ is unique.

We would like to stress that our findings need to be validated by results from other prospective studies, preferably with several studies pooled in order to obtain larger case numbers, stratified by sex. We can only speculate about the possible mechanisms underlying the observed associations. The positive association between acrylamide intake and the risk of colorectal tumors with an activating *KRAS* mutation, and within this endpoint the strongest association with G>T (an acrylamide-induced mutation in rodents), is compatible with glycidamide being a mutagen.

It is hypothesized that acrylamide may influence sex hormonal systems (10,36). The conspicuously lower incidence rate of CRC among women compared with men (37) is suggestive of sex hormonal involvement in its development. Decreased risks of CRC associated with hormone replacement therapy were reported in women (38). In addition, several prospective studies observed an inverse association

between oral contraceptive use and CRC risk (39). Little is known about the interaction between sex hormones and the *KRAS* and *APC* genes with respect to CRC development. There are some indications that estrogen receptors α and β protect against *APC*-dependent colorectal carcinogenesis (40), which would fit with the observed inverse association between acrylamide intake and a reduced risk of a tumor with a truncating *APC* mutation among women. In normal colonic mucosa, estrogen receptor β levels are higher in women than in men, whereas in tumors the level of this receptor is much more reduced compared with normal tissue in women than in men. This may indicate that this receptor, and thus the role of estrogenic substances, is more important in the etiology of CRC in women than in men (41).

Conclusions

In conclusion, this study showed a statistically significant positive association between acrylamide intake and the risk of colorectal tumors with, in particular, an activating mutation in the *KRAS* gene among men, and a statistically significant inverse association with the risk of tumors with a truncating mutation in the *APC* gene among women. Thus, acrylamide intake may influence the risk of CRC with activating *KRAS* mutations or truncating *APC* mutations in men and women differentially. However, a cautious interpretation of these results is necessary, since they are the first results considering tumor heterogeneity, because we performed many subgroup analyses, and because of the important differences in associations over the rather short follow-up period. We encourage replication of these associations in other prospective cohort studies with high-quality estimation of the acrylamide intake, e.g. studies using acrylamide and glycidamide to hemoglobin adducts as biomarkers of internal acrylamide exposure.

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References

1. Ferlay, J. *et al.* (2010) Estimates of cancer incidence and mortality in Europe in 2008. *Eur. J. Cancer*, **46**, 765–781.
2. IARC (1994) Some industrial chemicals. In: *IARC Monographs on the Evaluation of Carcinogen Risk to Humans*, Vol. 60. International Agency for Research on Cancer, Lyon.
3. Tareke, E. *et al.* (2002) Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.*, **50**, 4998–5006.
4. Friedman, M.A. *et al.* (1995) A lifetime oncogenicity study in rats with acrylamide. *Fundam. Appl. Toxicol.*, **27**, 95–105.
5. Johnson, K.A. *et al.* (1986) Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol. Appl. Pharmacol.*, **85**, 154–168.
6. Sumner, S.C. *et al.* (1999) Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chem. Res. Toxicol.*, **12**, 1110–1116.
7. Besarati Nia, A. *et al.* (2004) Genotoxicity of acrylamide and glycidamide. *J. Natl Cancer Inst.*, **96**, 1023–1029.
8. Besarati Nia, A. *et al.* (2005) DNA adduction and mutagenic properties of acrylamide. *Mutat. Res.*, **580**, 31–40.
9. Manjanatha, M.G. *et al.* (2006) Genotoxicity of acrylamide and its metabolite glycidamide administered in drinking water to male and female Big Blue mice. *Environ. Mol. Mutagen.*, **47**, 6–17.
10. Hogervorst, J.G. *et al.* (2010) The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. *Crit. Rev. Toxicol.*, **40**, 485–512.
11. Agrawal, A.K. *et al.* (1981) Neurotransmitter receptors in brain regions of acrylamide-treated rats. I: Effects of a single exposure to acrylamide. *Pharmacol. Biochem. Behav.*, **14**, 527–531.
12. Ali, S.F. *et al.* (1983) Effect of acrylamide on neurotransmitter metabolism and neuropeptide levels in several brain regions and upon circulating hormones. *Arch. Toxicol.*, **52**, 35–43.
13. Uphouse, L.L. *et al.* (1982) Interactions between “handling” and acrylamide on endocrine responses in rats. *Neurotoxicology*, **3**, 121–125.
14. Lee, T. *et al.* (2012) Expression analysis of hepatic mitochondria-related genes in mice exposed to acrylamide and glycidamide. *J. Toxicol. Environ. Health. A*, **75**, 324–339.
15. Mei, N. *et al.* (2008) Gene expression changes associated with xenobiotic metabolism pathways in mice exposed to acrylamide. *Environ. Mol. Mutagen.*, **49**, 741–745.
16. Clement, F.C. *et al.* (2007) Expression profile of human cells in culture exposed to glycidamide, a reactive metabolite of the heat-induced food carcinogen acrylamide. *Toxicology*, **240**, 111–124.
17. Mucci, L.A. *et al.* (2003) Dietary acrylamide and cancer of the large bowel, kidney, and bladder: absence of an association in a population-based study in Sweden. *Br. J. Cancer*, **88**, 84–89.
18. Larsson, S.C. *et al.* (2009) Dietary acrylamide intake and risk of colorectal cancer in a prospective cohort of men. *Eur. J. Cancer*, **45**, 513–516.
19. Mucci, L.A. *et al.* (2006) Prospective study of dietary acrylamide and risk of colorectal cancer among women. *Int. J. Cancer*, **118**, 169–173.
20. Pelucchi, C. *et al.* (2006) Dietary acrylamide and human cancer. *Int. J. Cancer*, **118**, 467–471.
21. Hogervorst, J.G. *et al.* (2008) Dietary acrylamide intake is not associated with gastrointestinal cancer risk. *J. Nutr.*, **138**, 2229–2236.
22. Fearon, E.R. *et al.* (1990) A genetic model for colorectal tumorigenesis. *Cell*, **61**, 759–767.
23. Kinzler, K.W. *et al.* (1996) Lessons from hereditary colorectal cancer. *Cell*, **87**, 159–170.
24. Fodde, R. *et al.* (2001) Disease model: familial adenomatous polyposis. *Trends Mol. Med.*, **7**, 369–373.
25. Miyaki, M. *et al.* (1994) Characteristics of somatic mutation of the adenomatous polyposis coli gene in colorectal tumors. *Cancer Res.*, **54**, 3011–3020.
26. Bos, J.L. (1989) Ras oncogenes in human cancer: a review. *Cancer Res.*, **49**, 4682–4689.
27. Lichtenborg, M. *et al.* (2004) APC mutations in sporadic colorectal carcinomas from The Netherlands Cohort Study. *Carcinogenesis*, **25**, 1219–1226.
28. van den Brandt, P.A. *et al.* (1990) A large-scale prospective cohort study on diet and cancer in The Netherlands. *J. Clin. Epidemiol.*, **43**, 285–295.
29. Van den Brandt, P.A. *et al.* (1990) Development of a record linkage protocol for use in the Dutch Cancer Registry for Epidemiological Research. *Int. J. Epidemiol.*, **19**, 553–558.
30. Brink, M. *et al.* (2003) K-ras oncogene mutations in sporadic colorectal cancer in The Netherlands Cohort Study. *Carcinogenesis*, **24**, 703–710.
31. Hogervorst, J.G. *et al.* (2007) A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol. Biomarkers Prev.*, **16**, 2304–2313.
32. Konings, E.J. *et al.* (2010) Validation of a database on acrylamide for use in epidemiological studies. *Eur. J. Clin. Nutr.*, **64**, 534–540.
33. Goldbohm, R.A. *et al.* (1994) Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. *Eur. J. Clin. Nutr.*, **48**, 253–265.
34. Goldbohm, R.A. *et al.* (1995) Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. *Eur. J. Clin. Nutr.*, **49**, 420–429.
35. Husøy, T. *et al.* (2008) Dietary exposure to 5-hydroxymethylfurfural from Norwegian food and correlations with urine metabolites of short-term exposure. *Food Chem. Toxicol.*, **46**, 3697–3702.
36. Shipp, A. *et al.* (2006) Acrylamide: review of toxicity data and dose-response analyses for cancer and noncancer effects. *Crit. Rev. Toxicol.*, **36**, 481–608.
37. Jemal, A. *et al.* (2011) Global cancer statistics. *CA Cancer J. Clin.*, **61**, 69–90.
38. Barnes, E.L. *et al.* (2012) Colorectal cancer in women: hormone replacement therapy and chemoprevention. *Climacteric*, **15**, 250–255.
39. Bosetti, C. *et al.* (2009) Oral contraceptives and colorectal cancer risk: a systematic review and meta-analysis. *Hum. Reprod. Update*, **15**, 489–498.
40. Cho, N.L. *et al.* (2007) Estrogen receptors alpha and beta are inhibitory modifiers of Apc-dependent tumorigenesis in the proximal colon of Min/+ mice. *Cancer Res.*, **67**, 2366–2372.
41. Campbell-Thompson, M. *et al.* (2001) Expression of estrogen receptor (ER) subtypes and ERbeta isoforms in colon cancer. *Cancer Res.*, **61**, 632–640.

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The influence of single nucleotide polymorphisms on the association between dietary acrylamide intake and endometrial cancer risk

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It is unclear whether the association between dietary acrylamide intake and endometrial cancer risk as observed in some epidemiological studies reflects a causal relationship. We aimed at clarifying the causality by analyzing acrylamide-gene interactions for endometrial cancer risk. The prospective Netherlands Cohort Study on diet and cancer includes 62,573 women, aged 55–69 years. At baseline, a random subcohort of 2589 women was selected for a case cohort analysis approach. Acrylamide intake of subcohort members and endometrial cancer cases ($n = 315$) was assessed with a food frequency questionnaire. Single nucleotide polymorphisms (SNPs) in genes in acrylamide metabolism, sex steroid systems, oxidative stress and DNA repair were assessed through a MassARRAY iPLEX Platform. Interaction between acrylamide and SNPs was assessed with Cox proportional hazards analysis, based on 11.3 years of follow-up. Among the results for 57 SNPs and 2 gene deletions, there were no statistically significant interactions after adjustment for multiple testing. However, there were nominally statistically significant interactions for SNPs in acrylamide-metabolizing enzymes: CYP2E1 (rs915906 and rs2480258) and the deletions of *GSTM1* and *GSTT1*. Although in need of confirmation, the interactions between acrylamide intake and CYP2E1 SNPs contribute to the evidence for a causal relationship between acrylamide and endometrial cancer risk.

Acrylamide is a probable human carcinogen (IARC class 2A; based on rodent studies) that was discovered in 2002 in various heat-treated carbohydrate-rich foods, such as cookies, potato chips, French fries and coffee. Since then, epidemiological studies have been performed in order to investigate the impact of dietary acrylamide intake on human cancer risks. The results of these studies are inconsistent: for some cancers (endometrial, ovarian, breast and kidney cancer) increased risks have been observed in some studies but not all. A recent meta-analysis on the association between acrylamide intake and endometrial cancer risk shows a pooled relative risk for high vs. low intake of 1.39 (95% CI 1.09–1.77) in never-smokers¹. In the most recent risk assessment of acrylamide, by the European Food Safety Authority (EFSA)², the epidemiological findings on acrylamide and cancer risk are discussed but not incorporated in the actual risk assessment. The most important reason for this is that the causality of the observed associations is still unclear, mainly due to the inconsistent associations across studies.

It is important to get more clarity on the causality of the observed epidemiological associations. They indicate that risks may be present at current dietary intake levels and they are still an underestimation of the true risk because of random measurement error of the acrylamide intake. In addition, the observed risks are considerably higher than predicted from rodent studies³. Moreover, virtually everyone is exposed to acrylamide through diet.

It is generally thought that acrylamide may cause cancer through the genotoxic action of acrylamide's metabolite glycidamide (generated by the action of cytochrome P4502E1 (CYP2E1)) but other mechanisms, such as effects on sex hormones, are hypothesized as well².

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Variable	Endometrial cancer cases	Subcohort
n*	364	1474
<i>Dietary variables</i>		
Acrylamide intake, µg/day	21.3 (12.7)	20.9 (11.7)
Coffee, g/day	488 (242)	496 (244)
Dutch spiced cake, g/day	6.0 (9.9)	5.6 (9.3)
Cookies, g/day	14.0 (10.8)	13.8 (10.5)
Potato crisps, g/day	0.38 (1.48)	0.39 (1.87)
French fries, g/day	4.0 (9.4)	3.7 (8.1)
Total energy intake, kcal	1671 (420)	1691 (399)
<i>Non-dietary variables</i>		
Age, yrs	61.3 (4.2)	61.5 (4.3)
BMI, kg/m ²	26.4 (4.2)	25.1 (3.6)
Age at menarche, yrs	13.4	13.7
Age at menopause, yrs	50.1	49.1
Parity, n children	2.2	2.8
n cigarettes per day	3.8 (7.0)	4.5 (7.6)
n smoking years	9.2 (14.5)	11.2 (15.7)
<i>Cigarette smoking status %</i>		
Never smokers	64.3	58.9
Former smokers	19.5	20.7
Current smokers	16.2	20.4
Ever use of postmenopausal hormone treatment, % yes	14.5	12.3
Ever use of oral contraceptives, % yes	13.9	24.8
Family history of endometrial cancer, % yes	4.1	3.0

Table 1. Characteristics of subcohort and endometrial cancer cases. *n represents number of subcohort members or cases after exclusion of participants with prevalent cancer at baseline, hysterectomy, incomplete or inconsistent dietary data, and a sample call rate <95%. The number of missing values varies for the variables in this Table.

In the present study, we investigated whether genetic make-up modifies the association between dietary acrylamide intake as assessed through a validated food frequency questionnaire and endometrial cancer risk, thereby contributing to evidence on acrylamide's mechanism of action and the causality of the observed associations between acrylamide and endometrial cancer risk. We selected SNPs in candidate genes involved in acrylamide metabolism (*CYP2E1*, *glutathione-s-transferases*, and *epoxide hydrolase*) and in mechanisms through which acrylamide is hypothesized to cause cancer: mechanisms involving DNA damage, sex hormones, and oxidative stress⁴. We specifically also look at never-smokers because cigarette smoke is an important source of acrylamide exposure¹.

Results

Table 1 shows the characteristics of the subcohort and endometrial cancer cases at baseline. Cases were more often never-smokers, smoked fewer cigarettes per day and for a shorter duration. They more often ever used postmenopausal hormone treatment and considerably less often ever used oral contraceptives. Cases had a lower age at menarche and a later age at menopause and they had fewer children. In addition, cases had a considerably higher BMI and more often a family history of endometrial cancer.

Main effect of acrylamide. There was no main effect of acrylamide with 20.3 years of follow-up (HR of highest versus the lowest quintile of intake: 1.03 (95% CI 0.71–1.51) and 0.98 (0.88–1.10) per 10 µg/day increment of intake (Table 2). A similar null association was observed for never-smokers. We decided to further focus on acrylamide-gene interactions for the first 11.3 years of follow-up period, for which we did see an association between acrylamide intake and endometrial cancer risk⁵.

Main effect of SNPs. Table 3 lists the SNPs that showed a (borderline) statistically significant association with endometrial cancer risk (11.3 years of follow-up). Women with variant alleles of rs1056827 in *CYP1B1*, rs944722 in *NOS2*, and rs2228000 in *XPC* showed a decrease in risk (p-trend 0.04, 0.05, 0.06, respectively). Women with variant alleles of rs2472299 in *CYP1A2*, rs3219489 in *MUTYH*, rs660149 in *PGR*, and rs1042157 and rs6839 in *SULT1A1* showed a positive trend over the number of variant alleles (p-trend 0.05, 0.09, 0.05, 0.07 and 0.07, respectively). A decreased risk of endometrial cancer was observed in women with a homozygous deletion of the *GSTM1* gene when both SNP selected to represent the deletion were not called: HR: 0.80 (0.58–1.11). The association was similar when the deletion was based on missing calls in rs10857795 alone and there was a statistically significant decrease in endometrial cancer risk when the deletion was based on rs200184852 alone (HR 0.71 (0.52–0.96)). However, none of the SNPs was statistically significantly associated with endometrial

Main association acrylamide*								
	N cases	Per 10 µg/day increment	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P trend
All women, 20.3 yrs FU	393	0.98 (0.88–1.10)	Ref (1.00)	0.87 (0.60–1.27)	0.86 (0.58–1.28)	0.95 (0.64–1.41)	1.03 (0.71–1.51)	0.77
All women, 1 st 11.3 yrs FU	221	1.05 (0.92–1.19)	Ref (1.00)	0.98 (0.60–1.59)	1.05 (0.63–1.74)	1.35 (0.82–2.22)	1.36 (0.84–2.19)	0.10
Never-smokers, 20.3 yrs FU	260	1.03 (0.90–1.18)	Ref (1.00)	1.07 (0.67–1.70)	1.14 (0.70–1.86)	1.08 (0.66–1.77)	1.44 (0.90–2.28)	0.17
Never-smokers, 1 st 11.3 yrs FU	150	1.13 (0.96–1.33)	Ref (1.00)	1.24 (0.66–2.31)	1.62 (0.87–3.03)	1.56 (0.83–2.92)	2.14 (1.20–3.82)	0.01

Table 2. Main associations between acrylamide intake and endometrial cancer risk. *Adjusted for age (yrs), age at menarche (yrs), age at menopause (yrs), parity (n children), ever use of oral contraceptives (yes/no), ever use of postmenopausal hormone use (yes/no), BMI (kg/m²), (kcal/day) and in the analyses for all women: current smoking (yes/no), quantity of smoking (cigarettes/day), duration of smoking (n smoking years), family history of endometrial cancer (yes/no), energy intake (kcal/day).

cancer risk after adjustment for multiple comparisons; none of the Benjamini-Hochberg FDR values were lower than the chosen 0.20 threshold.

Interactions between acrylamide intake and SNPs. None of the SNPs showed a statistically significant multiplicative interaction with acrylamide for the 11.3 year follow-up period when adjusted for multiple comparisons. In Table 4, we show interactions with SNPs in genes involved in acrylamide metabolism that are interesting because they have a higher *a priori* probability of modifying the association between acrylamide and cancer risk than the other selected SNPs because they determine internal exposure to acrylamide and glycidamide.

We observed nominally (borderline) statistically significant multiplicative interaction for 2 SNPs in *CYP2E1*: rs915906 for all women (p-interaction = 0.02) and for never-smoking women (p-interaction = 0.07), and rs2480258 for all women (p-interaction = 0.03). There only was an association between acrylamide and endometrial cancer risk in homozygous wild types for both SNPs.

We observed nominally statistically significant multiplicative interaction for the deletion of *GSTT1* for all women (p-interaction < 0.001) and never-smoking women (p-interaction = 0.02), although based on few cases with a homozygous deletion of *GSTT1* (12 among all women and 7 among never-smoking women). Acrylamide was only positively associated with endometrial cancer risk in women with at least one copy of the *GSTT1* allele. For the *GSTM1* gene deletion, the same pattern of associations was observed: the acrylamide-associated risk of endometrial cancer was only increased in women with at least one copy of the *GSTM1* gene but there was no statistically significant interaction with acrylamide intake. There were no interactions between acrylamide and SNPs in other acrylamide-metabolizing genes (*GSTA5*, *GSTP1* and *EPHX1*) (results not shown).

There were some (borderline) nominally statistically significant interactions between acrylamide and other SNPs (Supplemental Table 3): rs11252859 in *AKR1C1* (among never-smokers), rs1042157 and rs6839 in *SULT1A1*, rs3736599 in *SULT1E1* (among never-smokers), rs10432782 in *SOD1* (among never-smokers), rs3448 in *GPX1* (among never-smokers), rs1800566 in *NQO1* (among never-smokers), and rs2472299 in *CYP1A2* (among never-smokers). In addition, differences in the acrylamide dose-response relationship between the genotypes were observed for rs5275 in *PTGS2*, rs1280350 in *MGC12965*, rs1056836 in *CYP1B1*, rs2228000 in *XPC*, rs4986938 in *ESR2*, rs6428830 in *HSD3B1/B2* (among never-smokers) and rs64759180 in *RRM2* (Supplemental Table 3).

Discussion

The current study is the first to analyze acrylamide-gene interactions for (endometrial) cancer risk. We followed a candidate gene approach for identifying SNPs in genes involved in acrylamide metabolism and genes involved in the mechanisms by which acrylamide might cause cancer: a sex hormonal effect, oxidative stress and DNA damage.

The positive association between acrylamide intake and endometrial cancer risk that we observed previously after 11.3 years of follow-up⁵ was not present after 20.3 years of follow-up. A possible explanation for this is that the positive association observed in the first follow-up period was a spurious finding, making the current acrylamide-gene interaction analysis all the more relevant. Another possible explanation is that the baseline assessment of dietary acrylamide in 1986 is insufficiently representative of the dietary acrylamide intake in the etiologically relevant exposure period for endometrial cancers occurring in the latter half of the follow-up period. For this reason, we focused on the interaction between acrylamide and SNPs in the first 11.3 years of follow-up.

Although there were several SNPs showing a statistically significant interaction with acrylamide intake, none withstood the adjustment for multiple comparisons. However, we observed some nominally statistically significant interactions with SNPs in genes involved in acrylamide metabolism, thus having a higher *a priori* probability of modifying the association between acrylamide and cancer risk than the other selected SNPs.

Glycidamide (formed by epoxidation of acrylamide through *CYP2E1*) is often thought to be the compound responsible for acrylamide-induced carcinogenesis due to its genotoxicity. Therefore, studying the modifying effect of SNPs in *CYP2E1* on the association between acrylamide and cancer risk contributes important information on the causality of the association. We observed nominally statistically significant multiplicative interaction

Main association SNPs [†]									Benjamini-Hochberg p value
SNP	Total n cases	1 or 2 variant alleles vs homozygous wild type		1 variant allele vs homozygous wild type		2 variant alleles vs homozygous wild type		P trend per allele	
		n cases	HR (95% CI)	n cases	HR (95% CI)	n cases	HR (95% CI)		
CYP1A2, rs2472299	205	115	1.35 (1.01–1.81)	94	1.33 (0.98–1.81)	21	1.44 (0.86–2.40)	0.05	0.60
CYP1B1, rs1056827	203	86	0.86 (0.64–1.16)	79	0.94 (0.69–1.28)	7	0.44 (0.20–0.98)	0.09	0.62
MUTYH, rs3219489	205	98	1.27 (0.95–1.70)	82	1.24 (0.92–1.69)	16	1.42 (0.81–2.51)	0.09	0.62
NOS2, rs944722	198	115	0.81 (0.60–1.10)	92	0.89 (0.65–1.22)	23	0.61 (0.37–0.99)	0.05	0.60
PGR, rs660149	205	113	1.45 (1.08–1.95)	101	1.53 (1.13–2.07)	12	1.04 (0.55–1.97)	0.05	0.60
SULT1A1, rs1042157	205	141	1.31 (0.96–1.80)	105	1.27 (0.92–1.77)	36	1.44 (0.93–2.23)	0.07	0.60
SULT1A1, rs6839	205	127	1.29 (0.96–1.74)	93	1.24 (0.90–1.71)	34	1.44 (0.94–2.22)	0.07	0.60
XPC, rs2228000	205	81	0.78 (0.58–1.05)	70	0.81 (0.60–1.11)	11	0.61 (0.32–1.16)	0.06	0.60
GSTM1 deletion									
	Total n cases	Homozygous deleted vs 1 or 2 copies		P for HR	Benjamini-Hochberg p value				
		n cases	HR (95% CI)						
Deletion based on both GSTM1 SNPs	205	55	0.80 (0.58–1.11)						
Deletion based on rs10857795	205	64	0.80 (0.59–1.10)						
Deletion based on rs200184852	205	72	0.71 (0.52–0.96)	0.03 [‡]	0.60				

Table 3. Genetic variants showing a (borderline) statistically significant association with endometrial cancer risk, 11.3 years of follow-up. [†]Adjusted for age. [‡]P value for *GSTM1* deletion as assessed by missing call in rs200184852.

for 2 SNPs in *CYP2E1*: rs915906 and rs2480258. These 2 *CYP2E1* SNPs are in the intronic region of the gene and thus do not affect the protein code, but they may be in linkage disequilibrium with variants that are causative. It was shown that the allelic variants of both genes and specifically their combination were associated with an increase in micronuclei (MN) count in binucleated lymphocytes, a marker of DNA damage and an established risk marker for carcinogenesis⁶. *CYP2E1* metabolizes several other compounds than acrylamide, e.g., ethanol, benzene, nitrosamines, and acetaminophen⁷, and the enzyme bioactivates these compounds and thus increases their MN-forming potential. The observed increase in MN count observed with the variant alleles thus suggests increased *CYP2E1* activity of the variant alleles or alleles in linkage disequilibrium with these alleles. This then would suggest that acrylamide itself is the causative compound in endometrial carcinogenesis, because the strongest association between acrylamide and endometrial cancer risk was observed in homozygous wild types.

We also studied another SNP in *CYP2E1* (rs6413432), which did not show a statistically significant interaction with acrylamide intake but among never-smoking women, the risk of endometrial cancer was considerably higher in the homozygous wild types than in women with variant alleles: HRs per 10 µg/day increment in acrylamide intake were 1.25 (95% CI: 1.04–1.50, n = 95) and 1.06 (95% CI: 0.61–1.82, n = 25) for homozygous wild types versus women with variant alleles, respectively. This result points in the same hypothesized direction of acrylamide itself being the more relevant compound as the result for the other 2 SNPs because the variant allele of rs6413432 leads to increased *CYP2E1* gene expression⁸ and the association between acrylamide and endometrial cancer risk was strongest in women homozygous for the wild type allele.

The observed interaction with these *CYP2E1* SNPs contributes to the evidence for a causal association between acrylamide and endometrial cancer risk. Acrylamide has a high affinity for binding to thiol groups in proteins. Its effect on the nervous system is hypothesized to occur through binding to and disruption of proteins involved in neurotransmission⁹. For neurotoxicity, it is hypothesized that acrylamide itself is mainly responsible because acrylamide has a higher affinity for binding to proteins than glycidamide¹⁰. Despite the fact that a lot of attention is given to the genotoxicity of acrylamide's metabolite glycidamide as the mechanism of action, it is also hypothesized that acrylamide causes cancer through other mechanisms, such as effects on sex hormones. Those mechanisms may involve disruption of key proteins, in which acrylamide itself could be the causative compound.

We observed that women with at least one copy of *GSTM1* and *GSTT1* were at an increased acrylamide-associated risk of endometrial cancer, which was contrary to what we expected. Both acrylamide and glycidamide are detoxified by conjugation to glutathione and urinary excretion of the mercapturic acid complexes¹¹. However, it is unclear if glutathione conjugation of acrylamide occurs non-enzymatically or through catalyzation by GSTs¹².

Gene, SNP*	Acrylamide, continuous intake	Acrylamide, tertiles of intake							P for interaction	
		Tertile 1		Tertile 2		Tertile 3		P for trend		
	10 µg/day	N cases	HR (95% CI)	N cases	HR (95% CI)	N cases	HR (95% CI)		Raw p	Benjamini- Hochberg p value
All women										
<i>CYP2E1</i> , rs915906 = 0	1.17 (1.01–1.35)	31	Ref (1.00)	38	1.28 (0.74–2.20)	58	1.90 (1.15–3.12)	0.01	0.02	0.83
<i>CYP2E1</i> , rs915906 = 1	0.75 (0.50–1.12)	21	Ref (1.00)	18	1.07 (0.49–2.34)	12	0.73 (0.30–1.76)	0.49		
Never-smokers										
<i>CYP2E1</i> , rs915906 = 0	1.34 (1.09–1.63)	21	Ref (1.00)	25	1.40 (0.71–2.75)	41	2.31 (1.26–4.21)	0.006	0.07	0.61
<i>CYP2E1</i> , rs915906 = 1	0.91 (0.55–1.49)	11	Ref (1.00)	13	1.70 (0.65–4.43)	9	1.21 (0.41–3.56)	0.74		
All women										
<i>CYP2E1</i> , rs2480258 = 0	1.22 (1.02–1.45)	28	Ref (1.00)	31	1.31 (0.72–2.37)	46	1.82 (1.06–3.11)	0.03	0.03	0.83
<i>CYP2E1</i> , rs2480258 = 1	0.88 (0.69–1.11)	24	Ref (1.00)	25	1.09 (0.56–2.12)	24	1.13 (0.57–2.23)	0.74		
Never-smokers										
<i>CYP2E1</i> , rs2480258 = 0	1.37 (1.10–1.72)	20	Ref (1.00)	21	1.44 (0.70–2.96)	34	2.24 (1.19–4.20)	0.01	0.11	0.70
<i>CYP2E1</i> , rs2480258 = 1	0.96 (0.71–1.31)	12	Ref (1.00)	17	1.70 (0.76–3.83)	16	1.56 (0.65–3.74)	0.34		
All women										
<i>GSTM1</i> 1 or 2 copies, all SNPs	1.12 (0.97–1.30)	36	Ref (1.00)	36	1.04 (0.60–1.81)	59	1.66 (1.00–2.74)	0.04	0.14 [†]	0.92
<i>GSTM1</i> deleted, all SNPs	0.90 (0.63–1.30)	16	Ref (1.00)	20	1.68 (0.71–3.99)	11	0.93 (0.39–2.21)	0.94		
Never-smokers										
<i>GSTM1</i> 1 or 2 copies, all SNPs	1.25 (1.05–1.49)	21	Ref (1.00)	24	1.52 (0.75–3.05)	42	2.56 (1.39–4.68)	0.002	0.28	0.86
<i>GSTM1</i> deleted, all SNPs	0.90 (0.53–1.54)	11	Ref (1.00)	14	1.78 (0.67–4.72)	8	0.78 (0.27–2.23)	0.73		
All women										
<i>GSTT1</i> 1 or 2 copies, all SNPs	1.13 (0.98–1.30)	48	Ref (1.00)	52	1.23 (0.78–1.93)	66	1.60 (1.04–2.44)	0.03	0.07	0.92
<i>GSTT1</i> deleted, all SNPs	0.55 (0.23–1.28)	4	Ref (1.00)	4	0.83 (0.13–5.41)	4	0.28 (0.03–2.77)	0.24		
Never-smokers										
<i>GSTT1</i> 1 or 2 copies, all SNPs	1.33 (1.10–1.61)	29	Ref (1.00)	35	1.56 (0.88–2.76)	49	2.35 (1.39–3.98)	0.001	0.02	0.61
<i>GSTT1</i> deleted, all SNPs	0.43 (0.19–0.97)	3	Ref (1.00)	3	0.83 (0.17–4.10)	1	0.13 (0.01–2.05)	0.08		

Table 4. Interactions between SNPs in genes in acrylamide metabolism and dietary acrylamide intake on the risk of endometrial cancer, 11.3 years of follow-up. Adjusted for age (yrs), age at menarche (yrs), age at menopause (yrs), parity (n children), ever use of oral contraceptives (yes/no), ever use of postmenopausal hormone use (yes/no), BMI (kg/m²), current smoking (yes/no), quantity of smoking (cigarettes/day), duration of smoking (n smoking years), family history of endometrial cancer (yes/no), energy intake (kcal/day). *0 = homozygous wild type, 1 = 1 or 2 variant alleles. [†]p for interaction borderline statistically significant (p = 0.09) when deletion was based on missing calls for rs200184852.

Interestingly, regardless of acrylamide intake, women with a double deletion of *GSTM1* were at a decreased risk of endometrial cancer in our study, which has been observed before¹³, and also for some other cancers^{14,15}. A possible explanation is that GSTs catalyze the conjugation of reduced glutathione (GSH) to compounds that protect against endometrial cancer or that they bioactivate compounds involved in endometrial carcinogenesis, for instance catechol estrogens¹⁶. In addition, conjugation of acrylamide with GSH can result in depletion of cellular GSH stores, leading to an altered redox status of the cell. This can affect gene expression directly or through regulating various redox-dependent transcription factors⁴. Considering the fact that acrylamide induces GST activity^{17,18}, it would be expected that the positive association between acrylamide and endometrial cancer is only present among women in whom the activity of GST can be induced; *i.e.* women with at least one copy of the genes.

An interesting observation in this context is that in a study on 85 persons of whom 51 were occupationally exposed to acrylamide, persons with the *GSTM1* null genotype had lower urinary levels of the mercapturic acid metabolite of acrylamide in combination with a higher ratio of the glycidamide mercapturic acid metabolite to the acrylamide mercapturic acid metabolite than *GSTM1* positive persons¹⁹. The authors speculate that this

indicates that in persons with the *GSTM1* null genotype a higher percentage of acrylamide is converted to glycidamide. In combination with the fact that we only observed an association between acrylamide and endometrial cancer risk in women with at least one copy of *GSTM1*, this could, in line with the results for the *CYP2E1* SNPs, suggest that acrylamide itself is the causative compound in endometrial carcinogenesis. Whatever the biological explanation behind the observed interactions with *GSTs*, it is remarkable that *GSTM1* and *GSTT1* show a similar interaction pattern.

There were some (borderline) nominally statistically significant interactions between acrylamide and other SNPs: rs11252859 in *AKR1C1*, rs1042157 and rs6839 in *SULT1A1*, rs3736599 in *SULT1E1*, rs10432782 in *SOD1*, rs3448 in *GPX1*, rs1800566 in *NQO1*, and rs2472299 in *CYP1A2*. In addition, differences in the acrylamide dose-response relationship between the genotypes were observed for rs5275 in *PTGS2*, rs1280350 in *MGC12965*, rs1056836 in *CYP1B1*, rs2228000 in *XPC*, rs4986938 in *ESR2*, rs6428830 in *HSD3B1/B2* and rs64759180 in *RRM2*. For all these SNPs it is even more important that the interaction between acrylamide intake and these SNPs is first corroborated or refuted in other studies in order to be able to judge whether our findings were chance findings or not. Therefore it is premature to elaborately discuss their possible role in acrylamide-induced endometrial carcinogenesis here.

This study has some limitations. Acrylamide intake was only assessed once, at baseline. The association between acrylamide and endometrial cancer risk was only present in the first half of the 20.3 year follow-up period, possibly due to the fact that the single dietary intake measurement was not representative of the relevant exposure of the later cases. Using the full 20.3 year follow-up period for analysis (results not shown), there were some similar nominally statistically significant interactions as with the 11.3 year follow-up period, namely with rs6839 (*SULT1A1*), rs2472299 (*CYP1A2*), and rsrs3448 (*GPX1*). However, the differences between the genotypes were not as clear as with the 11.3 year follow-up period. The other statistically significant interactions that were observed with 11.3 years of follow-up were not statistically significant with 20.3 years of follow-up. With 20.3 years of follow-up, there were some statistically significant interactions that were not observed with 11.3 years of follow-up: rs11252887 (*AKR1C1*) (only women with 1 or 2 variant alleles showed a clear increase in acrylamide-associated endometrial cancer risk), rs28362491 (*NFKB1*) (increased acrylamide-associated risk only in homozygous wild types), rs2228000 (*XPC*) (increased acrylamide-associated risk only in never-smoking homozygous wild types) and rs5275 (*PTGS2*) (increased acrylamide-associated risk only in homozygous wild types). None of the interactions were statistically significant after adjustment for multiple testing.

Rs2480258 in *CYP2E1* that statistically significantly modified the association between acrylamide intake and endometrial cancer risk was not in Hardy-Weinberg equilibrium, although the deviation was minor ($p = 0.03$) and not statistically significant after adjustment for multiple testing. This may indicate that the genotypes for this SNP were measured with some error. However, there is no reason to assume that this error is different for cases and subcohort members or for different categories of acrylamide intake. Therefore, this potential genotyping error would rather have led to missing a true interaction (if any) than detecting an interaction²⁰.

Some of the interactions that we discussed may be chance findings, considering that none of the interactions withstood the adjustment for multiple comparisons. However, finding interactions for multiple SNPs in the same gene for *CYP2E1* decreases the likelihood that they are chance findings, especially when there are clear differences in the dose-response pattern of acrylamide between the genotypes.

Both the homozygous deletion of *GSTM1* and that of *GSTT1* in our population (based on the combination of SNPs selected for these genes) were low (31% and 8%, respectively) compared to the reported prevalence in Caucasian populations (40–60% for *GSTM1* and 10–20% for *GSTT1*). In a PCR study (not shown), we tested some of the samples ($n = 33$) that showed a discrepancy in the iPLEX assay between rs10857795 and rs200184852 to represent the *GSTM1* deletion and rs4630 and rs1040309 to represent the *GSTT1* deletion ($n = 37$). All the samples that had no call for rs200184852 (but did have a call for rs10857795) in the iPLEX assay showed absence of a PCR product in the PCR study (results not shown). Only 51% of the samples that had no call for rs4630 (but did have a call for rs1040309) in the iPLEX assay showed absence of a PCR product in the PCR study. Thus, it can be assumed that the percentage of study participants with a deletion of *GSTM1* is closer to 42% (as reflected by rs200184852) than to the 31% reflected by both *GSTM1* SNPs. For *GSTT1*, it cannot be concluded which SNP best represents absence of the deletion but the true percentage is probably somewhere between 11% (rs104003609) and 15% (rs4630). In conclusion, the percentages of the *GST* deletions in this study are within the ranges of published percentages for Caucasian populations.

Strengths of this study are the prospective nature, the complete follow-up, and the fact that we observed a main effect of acrylamide, which may mean that it was assessed reasonably well in this study.

In conclusion, when we adjusted for multiple comparisons, there was no statistically significant interaction between SNPs and acrylamide intake for endometrial cancer risk. However, the nominally statistically significant interaction between acrylamide and SNPs in *CYP2E1* contributes to the evidence of a causal association between acrylamide intake and endometrial cancer risk but confirmation is needed. Based on this study, we recommend prospective cohort studies on acrylamide-gene interactions and for some genes in particular: *CYP2E1* and *GSTs*. These studies are preferably larger than the present study.

Methods

Study Cohort, Cases and Follow-up. The Netherlands Cohort Study on diet and cancer began in September 1986 with the inclusion of 62,573 women aged 55–69 years. Data on dietary habits and other risk factors were collected through a self-administered questionnaire at baseline in 1986. In addition, 75% of the participants sent in toenail clippings. Participants gave informed consent by returning the completed questionnaire. The NLCS, using toenail DNA for genotyping, and associated protocols were approved by the review boards of TNO

Nutrition and Food Research (Zeist, the Netherlands) and Maastricht University (Maastricht, the Netherlands). All methods were applied according to the approved guidelines.

Following the case-cohort approach, cases were enumerated for the entire cohort, while the accumulated person-time at risk for the full cohort was estimated from a random subcohort of 2589 women. Since baseline, the subcohort has been followed up regularly for vital status information. Incident cases in the full cohort were detected by annual computerized record linkages to the regional cancer registries and the Netherlands Pathology Registry. Further details on design and methods of follow-up are presented elsewhere^{21–24}.

After 20.3 years of follow-up (Sept. 1986–Dec. 2006), there were 588 microscopically confirmed primary carcinomas of the endometrium ([ICD-O]-3:C54). Cohort members were excluded from analysis if their dietary data were incomplete or inconsistent, they had not sent in toenail clippings, they had no or inferior (call rate <95%) data on SNPs, or if they reported to have had a hysterectomy. Figure 1 shows the selection and exclusion steps that resulted in the numbers of cases and subcohort members available for analysis.

Acrylamide Intake Assessment. A food frequency questionnaire with questions on 150 food items was used for estimating dietary habits. The acrylamide intake was estimated from the mean acrylamide level of foods on the Dutch market, and the frequency of consumption and portion size of the foods, as described in detail elsewhere⁵.

Selection of genes and SNPs. The selection of genes focused on genes involved in 1) acrylamide metabolism (*CYP2E1*, *GSTs* and *EPHX1*) and 2) the hypothesized mechanisms of acrylamide-induced carcinogenesis:⁴ 2a) a sex hormonal effect (involving sex hormone synthesis/metabolism or sex hormone nuclear receptors); 2b) oxidative stress; 2c) genotoxicity (DNA repair); or 2d) genes, not belonging to 1 or 2a–c, that were shown to be significant in an acrylamide-related polymorphism study^{19,25–28} or because they are in genes that were shown differentially expressed upon acrylamide exposure in acrylamide-related gene expression studies^{17,18,29–40}.

Genes and SNPs of interest were identified from the literature (HugeNavigator and PubMed) and from a personal communication (for SNP rs1280350 in *MGC12965*) with Jos Kleijnans (Dept. of Toxicogenomics, Maastricht University). This latter SNP was shown to be associated with the level of acrylamide-hemoglobin adducts in cord blood of newborns in the Newborns and Genotoxic exposure risks (NewGeneris) project.

Preferably SNPs shown to be associated with a cancer involving sex hormones (endometrial, ovarian, breast and prostate cancer) were selected. However, we also selected some SNPs with no literature on their relation with the cancers of interest but that were shown to be have an association or effect in the above-mentioned acrylamide-related polymorphism study^{19,25–28} or gene expression studies^{17,18,29–40}. It is unsure if *in vitro* or *in vivo* animal gene expression studies can be extrapolated to humans but at least these studies give indications that acrylamide exposure may involve effects on or interfere with these genes/enzymes.

Only validated SNPs with a minor allele frequency $\geq 10\%$ in dbSNP (Caucasians) were selected. The functionality of the SNPs (as based on the F-value in F-SNP)⁴¹ and the region of the SNP in the gene were no selection criteria per se but they were used to choose between SNPs when there were many interesting SNPs per gene.

There were too many potentially interesting genes (see Supplemental Table 1), so we prioritized SNPs in acrylamide-metabolizing genes and (SNPs in) genes that showed an association or effect in acrylamide studies on gene polymorphisms and gene expression changes.

GSTM1 and *GSTT1* are genes that are completely deleted in a large proportion of the population. The beginning and ending of the deleted sequences of *GSTM1* and *GSTT1* are not precisely known. Thus, it was impossible to design 1 assay (based on single base extension) for the deletion, as is done for SNPs. Therefore, we chose 3 SNPs in *GSTM1* and *GSTT1* each to represent the deletions (see Supplemental Table 1); when all 3 SNPs were not called, we assumed deletion of the gene.

66 SNPs were designed to fit together onto the 2 multiplexes that we budgeted: 6 SNPs to determine the *GST* deletions and 60 SNPs in other genes, see Supplemental Table 2.

DNA isolation and genotyping. DNA was isolated from 15 mg of toenail clippings, following a protocol described elsewhere⁴². Genotyping was performed by Agena, on the MassARRAY platform using the iPLEX TM assay⁴³. This method has been used before to successfully genotype DNA from toenails^{42,44,45}.

5% of the samples ($n = 190$) were duplicate samples to check the reproducibility of genotyping, which was >99%. Supplemental Table 2 shows the 60 SNPs that were analyzed. Three of the 60 SNPs that were genotyped had a call rate <80% and were excluded from the analyses. Six SNPs out of the remaining 57 SNPs did not adhere to Hardy-Weinberg equilibrium ($p < 0.05$). We excluded samples with a call rate <95% (18 cancer cases, 76 subcohort members). With regard to the SNPs selected to represent the *GSTM1* deletion, rs10857795 was not called in 36%, rs200184852 in 42% and rs74837985 in only 2% of the subcohort. The latter value appears to be due to genotyping error. Therefore, we decided to base the assessment of the deletion of the *GSTM1* gene only on rs10857795 and rs200184852. 31% had a missing value for both rs10857795 and rs200184852. With regard to *GSTT1*, rs2844008 was not called in 58%, rs4630 in 15%, and rs140309 in 11% of the subcohort. 8% had a missing value for all 3 *GSTT1* SNPs.

Statistical Analysis. Hazard rate ratios (HRs) and 95% confidence intervals were obtained through Cox proportional hazards regression with STATA software (package 13), with standard errors estimated using the robust Huber-White sandwich estimator to account for additional variance introduced by sampling from the cohort. The proportional hazards assumption was tested using scaled Schoenfeld residuals.

Covariables, selected from the literature, for the models of the main effect of acrylamide and acrylamide-gene interactions were: age, body mass index, age at menarche, age at menopause, ever use of oral contraceptives,

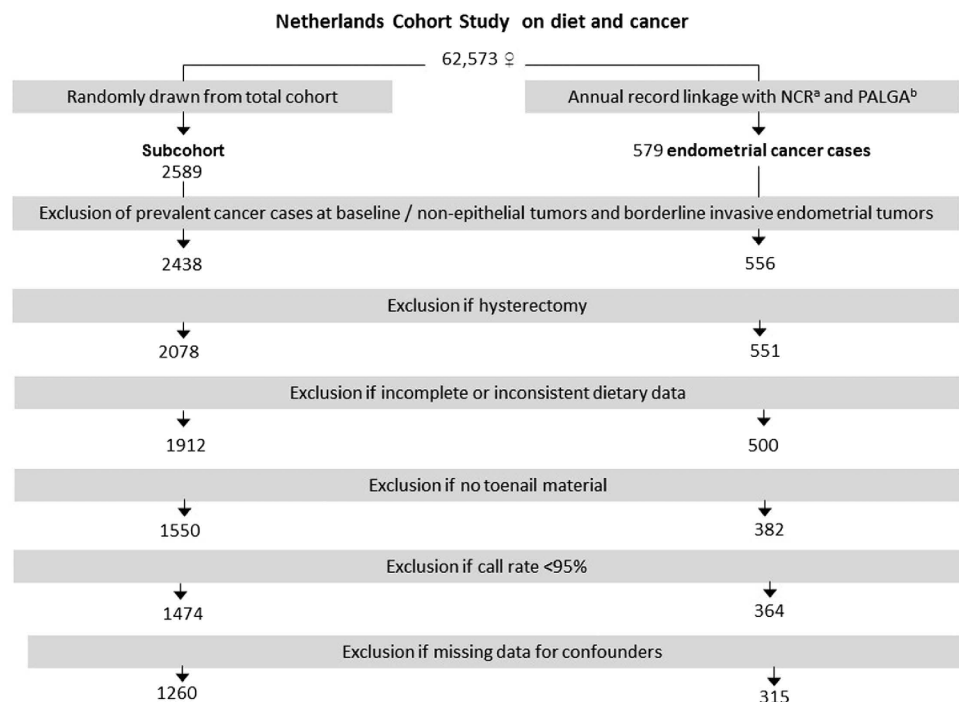


Figure 1. Flow chart of subcohort members and endometrial cancer cases. ^aNCR = Netherlands Cancer Registry. ^bPALGA = Dutch Pathology Registry.

parity, ever use of postmenopausal hormone, family history of endometrial cancer, and energy intake. Smoking status, the duration of smoking and the number of cigarettes per day were included in the model, because cigarette smoke is an important source of acrylamide. Smokers have been shown to have on average four times higher exposure to acrylamide than non-smokers⁴⁶. Moreover, smoking is inversely associated with endometrial cancer risk⁴⁷. Therefore, subgroup analyses for never-smokers were performed. The main associations between SNPs and endometrial cancer risk were adjusted for age only.

In a previous analysis, we observed a positive main effect of acrylamide intake on endometrial cancer risk after 11.3 years of follow-up⁵. In the present study, our first step was to investigate whether this main effect was also present with 20.3 years of follow-up.

Multiplicative interaction between acrylamide intake and SNPs was tested using product terms of the continuous acrylamide intake variable and genotype. For statistical power reasons, we used a dominant genetic model (i.e., 1 or 2 variant alleles versus homozygous wild type). Tests for acrylamide dose-response trends in genotype strata were performed by fitting the mean acrylamide intake in the tertiles as a continuous variable.

We applied the False Discovery Rate method developed by Benjamini-Hochberg to adjust for multiple testing⁴⁸ with the expected proportion of false positives set at 20%, which is applied regularly in candidate gene studies^{49,50}. We performed separate adjustment for multiple testing for all women and for never-smoking women.

Two-sided p values are reported throughout this paper.

References

1. Je, Y. Dietary acrylamide intake and risk of endometrial cancer in prospective cohort studies. *Arch. Gynecol. Obstet.* **291**, 1395–1401 (2015).
2. EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). Scientific Opinion on acrylamide in food. *EFSA Journal* **13**, 4104, 321pp (2015).
3. Mucci, L. A. & Adami, H. O. The role of epidemiology in understanding the relationship between dietary acrylamide and cancer risk in humans. *Adv. Exp. Med. Biol.* **561**, 39–47 (2005).
4. Besaratinia, A. & Pfeifer, G. P. A review of mechanisms of acrylamide carcinogenicity. *Carcinogenesis* **28**, 519–528 (2007).
5. Hogervorst, J. G., Schouten, L. J., Konings, E. J., Goldbohm, R. A. & van den Brandt, P. A. A Prospective Study of Dietary Acrylamide Intake and the Risk of Endometrial, Ovarian, and Breast Cancer. *Cancer Epidemiol. Biomarkers Prev.* **16**, 2304–2313 (2007).
6. Merlo, D. F. *et al.* Micronuclei in Cord Blood Lymphocytes and Associations with Biomarkers of Exposure to Carcinogens and Hormonally Active Factors, Gene Polymorphisms, and Gene Expression: The NewGeneris Cohort. *Environ. Health Perspect.* **122**, 193–200 (2014).
7. Cederbaum, A. I. CYP2E1—biochemical and toxicological aspects and role in alcohol-induced liver injury. *Mt. Sinai J. Med.* **73**, 657–672 (2006).
8. Uematsu, F. *et al.* Restriction fragment length polymorphism of the human CYP2E1 (cytochrome P450IIE1) gene and susceptibility to lung cancer: possible relevance to low smoking exposure. *Pharmacogenetics* **4**, 58–63 (1994).
9. LoPachin, R. M. & Gavin, T. Molecular mechanism of acrylamide neurotoxicity: lessons learned from organic chemistry. *Environ. Health Perspect.* **120**, 1650–1657 (2012).
10. Carere, A. Genotoxicity and carcinogenicity of acrylamide: a critical review. *Ann. Ist. Super. Sanita* **42**, 144–155 (2006).
11. Fennell, T. R. *et al.* Metabolism and hemoglobin adduct formation of acrylamide in humans. *Toxicol. Sci.* **85**, 447–459 (2005).

12. Doroshenko, O. *et al.* In vivo role of cytochrome P450 2E1 and glutathione-S-transferase activity for acrylamide toxicokinetics in humans. *Cancer Epidemiol. Biomarkers Prev.* **18**, 433–443 (2009).
13. Ashton, K. A. *et al.* Polymorphisms in genes of the steroid hormone biosynthesis and metabolism pathways and endometrial cancer risk. *Cancer Epidemiol.* **34**, 328–337 (2010).
14. Emeville, E. *et al.* Copy number variation of GSTT1 and GSTM1 and the risk of prostate cancer in a Caribbean population of African descent. *Plos one* **9**, e107275 (2014).
15. Roodi, N., Dupont, W. D., Moore, J. H. & Parl, F. F. Association of homozygous wild-type glutathione S-transferase M1 genotype with increased breast cancer risk. *Cancer Res.* **64**, 1233–1236 (2004).
16. Butterworth, M., Lau, S. S. & Monks, T. J. Formation of catechol estrogen glutathione conjugates and gamma-glutamyl transpeptidase-dependent nephrotoxicity of 17beta-estradiol in the golden Syrian hamster. *Carcinogenesis* **18**, 561–567 (1997).
17. Lee, T. *et al.* Expression analysis of hepatic mitochondria-related genes in mice exposed to acrylamide and glycidamide. *J. Toxicol. Environ. Health A* **75**, 324–339 (2012).
18. Sen, A., Ozgun, O., Arinc, E. & Arslan, S. Diverse action of acrylamide on cytochrome P450 and glutathione S-transferase isozyme activities, mRNA levels and protein levels in human hepatocarcinoma cells. *Cell. Biol. Toxicol.* **28**, 175–186 (2012).
19. Huang, Y. F. *et al.* Association of CYP2E1, GST and mEH genetic polymorphisms with urinary acrylamide metabolites in workers exposed to acrylamide. *Toxicol. Lett.* **10**, 118–126 (2011).
20. Fardo, D. W., Becker, K. D., Bertram, L., Tanzi, R. E. & Lange, C. Recovering unused information in genome-wide association studies: the benefit of analyzing SNPs out of Hardy-Weinberg equilibrium. *Eur. J. Hum. Genet.* **17**, 1676–1682 (2009).
21. van den Brandt, P. A. *et al.* A large-scale prospective cohort study on diet and cancer in The Netherlands. *J. Clin. Epidemiol.* **43**, 285–295 (1990).
22. van den Brandt, P. A., Schouten, L. J., Goldbohm, R. A., Dorant, E. & Hunen, P. M. Development of a record linkage protocol for use in the Dutch Cancer Registry for Epidemiological Research. *Int. J. Epidemiol.* **19**, 553–558 (1990).
23. Goldbohm, R. A. *et al.* Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. *Eur. J. Clin. Nutr.* **48**, 253–265 (1994).
24. Goldbohm, R. A. *et al.* Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. *Eur. J. Clin. Nutr.* **49**, 420–429 (1995).
25. Pingarilho, M. *et al.* Genetic polymorphisms in detoxification and DNA repair genes and susceptibility to glycidamide-induced DNA damage. *J. Toxicol. Environ. Health A* **75**, 920–933 (2012).
26. Duale, N. *et al.* Biomarkers of human exposure to acrylamide and relation to polymorphisms in metabolizing genes. *Toxicol. Sci.* **108**, 90–99 (2009).
27. Pingarilho, M. *et al.* Induction of sister chromatid exchange by acrylamide and glycidamide in human lymphocytes: role of polymorphisms in detoxification and DNA-repair genes in the genotoxicity of glycidamide. *Mutat. Res.* **752**, 1–7 (2013).
28. Kjuus, H. *et al.* Chromosome aberrations in tunnel workers exposed to acrylamide and N-methylolacrylamide. *Scand. J. Work Environ. Health* **31**, 300–306 (2005).
29. Clement, F. C., Dip, R. & Naegeli, H. Expression profile of human cells in culture exposed to glycidamide, a reactive metabolite of the heat-induced food carcinogen acrylamide. *Toxicology* **240**, 111–124 (2007).
30. Mei, N. *et al.* Gene expression changes associated with xenobiotic metabolism pathways in mice exposed to acrylamide. *Environ. Mol. Mutagen.* **49**, 741–745 (2008).
31. Camacho, L. *et al.* Effects of acrylamide exposure on serum hormones, gene expression, cell proliferation, and histopathology in male reproductive tissues of Fischer 344 rats. *Toxicol. Lett.* **211**, 135–143 (2012).
32. Ehlers, A. *et al.* Dose dependent molecular effects of acrylamide and glycidamide in human cancer cell lines and human primary hepatocytes. *Toxicol. Lett.* **217**, 111–120 (2013).
33. Hasegawa, K. *et al.* Acrylamide-responsive genes in the nematode *Caenorhabditis elegans*. *Toxicol. Sci.* **101**, 215–225 (2008).
34. Hochstenbach, K. *et al.* Global gene expression analysis in cord blood reveals gender-specific differences in response to carcinogenic exposure in utero. *Cancer Epidemiol. Biomarkers Prev.* **21**, 1756–1767 (2012).
35. Kim, K. Effect of subchronic acrylamide exposure on the expression of neuronal and inducible nitric oxide synthase in rat brain. *J. Biochem. Mol. Toxicol.* **19**, 162–168 (2005).
36. Lyn-Cook, L. E. Jr. *et al.* Food contaminant acrylamide increases expression of Cox-2 and nitric oxide synthase in breast epithelial cells. *Toxicol. Ind. Health* **27**, 11–118 (2011).
37. Sadek, I. A. Short-term studies of the effect of acrylamide on the testes of the Egyptian toad. *Folia Morphol. (Praha)* **37**, 427–430 (1989).
38. Shan, X. *et al.* Curcumin and (–)-epigallocatechin-3-gallate attenuate acrylamide-induced proliferation in HepG2 cells. *Food Chem. Toxicol.* **66**, 194–202 (2014).
39. Song, J. *et al.* Protection of cyanidin-3-glucoside against oxidative stress induced by acrylamide in human MDA-MB-231 cells. *Food Chem. Toxicol.* **58**, 306–310 (2013).
40. Yang, H. J. *et al.* Toxicological effects of acrylamide on rat testicular gene expression profile. *Reprod. Toxicol.* **19**, 527–534 (2005).
41. Lee, P. H. & Shatky, H. F-SNP: computationally predicted functional SNPs for disease association studies. *Nucl. Acids Res.* **36** (suppl 1), D820–D824, Database accessed on Dec 1 2013) (2008).
42. Hogervorst, J. G. *et al.* DNA from nails for genetic analyses in large-scale epidemiologic studies. *Cancer Epidemiol. Biomarkers Prev.* **23**, 2703–2712 (2014).
43. Gabriel, S., Ziaugra, L. & Tabbaa, D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr. Protoc. Hum. Genet* Chapter 2, Unit 2 12 (2009).
44. Geybels, M. S. *et al.* Selenoprotein gene variants, toenail selenium levels, and risk for advanced prostate cancer. *J. Natl. Cancer Inst.* **106**, dju003 (2014).
45. Deckers, I. A. *et al.* Polymorphisms in genes of the renin-angiotensin-aldosterone system and renal cell cancer risk: Interplay with hypertension and intakes of sodium, potassium and fluid. *Int. J. Cancer* **136**, 1104–1116 (2015).
46. Schettgen, T. *et al.* Deterioration of haemoglobin adducts of acrylamide and glycidamide in smoking and non-smoking persons of the general population. *Int. J. Hyg. Environ. Health* **207**, 531–539 (2004).
47. Al-Zoughool, M. *et al.* Risk of endometrial cancer in relationship to cigarette smoking: results from the EPIC study. *Int. J. Cancer* **121**, 2741–2747 (2007).
48. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Statist. Soc.* **57**, 289–300 (1995).
49. Geybels, M. S., van den Brandt, P. A., van Schooten, F. J. & Verhage, B. A. Oxidative stress-related genetic variants, pro- and antioxidant intake and status, and advanced prostate cancer risk. *Cancer Epidemiol. Biomarkers Prev.* **24**, 178–186 (2015).
50. Kim, C. *et al.* Genetic polymorphisms in oxidative stress pathway genes and modification of BMI and risk of non-Hodgkin lymphoma. *Cancer Epidemiol. Biomarkers Prev.* **21**, 866–868 (2012).

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Author Contributions

The author contributions were as follows: J.G.F.H. conceived the analyses, coordinated the genotyping, conducted the analyses, interpreted the results and wrote the manuscript; P.A.v.d.B. conceived, coordinates and supervises the Netherlands Cohort Study, and critically reviewed the manuscript; R.W.L.G. supervised the genotyping and critically reviewed the manuscript; F.-J.v.S. provided laboratory facilities for genotyping and critically reviewed the manuscript; and L.J.S. co-conceived the analyses, coordinated and supervised the genotyping, coordinates and supervises the data management for the Netherlands Cohort Study, and critically reviewed the manuscript. This study was funded by the Dutch Cancer Society (KWF), grant number: UM 2011-5123.

Additional Information

Supplementary information accompanies this paper at <http://www.nature.com/srep>

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Interactions between dietary acrylamide intake and genes for ovarian cancer risk

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Abstract Some epidemiological studies observed a positive association between dietary acrylamide intake and ovarian cancer risk but the causality needs to be substantiated. By analyzing gene-acrylamide interactions for ovarian cancer risk for the first time, we aimed to contribute to this. The prospective Netherlands Cohort Study on diet and cancer includes 62,573 women, aged 55–69 years. At baseline in 1986, a random subcohort of 2589 women was sampled from the total cohort for a case cohort analysis approach. Dietary acrylamide intake of subcohort members and ovarian cancer cases ($n = 252$, based on 20.3 years of follow-up) was assessed with a food frequency questionnaire. We selected single nucleotide polymorphisms (SNPs) in genes in acrylamide metabolism and in genes involved in the possible mechanisms of acrylamide-induced carcinogenesis (effects on sex steroid systems, oxidative stress and DNA damage). Genotyping was done on DNA from toenails through Agena's MassARRAY iPLEX platform. Multiplicative interaction between acrylamide intake and SNPs was assessed with

Cox proportional hazards analysis. Among the results for 57 SNPs and 2 gene deletions, there were no statistically significant interactions between acrylamide and gene variants after adjustment for multiple testing. However, there were several nominally statistically significant interactions between acrylamide intake and SNPs in the *HSD3B1/B2* gene cluster: (rs4659175 (p interaction = 0.04), rs10923823 (p interaction = 0.06) and its proxy rs7546652 (p interaction = 0.05), rs1047303 (p interaction = 0.005), and rs6428830 (p interaction = 0.05). Although in need of confirmation, results of this study suggest that acrylamide may cause ovarian cancer through effects on sex hormones.

Keywords Dietary acrylamide · Single nucleotide polymorphism · Ovarian cancer · Prospective cohort

Introduction

Acrylamide, a probable human carcinogen (IARC class 2A; based on rodent studies), was discovered in 2002 in various heat-treated carbohydrate-rich foods, such as cookies, potato chips, French fries and coffee. Since then, epidemiological studies have been performed in order to investigate the impact of dietary acrylamide intake on human cancer risks. The results of these studies are inconsistent: for some cancers (endometrial, ovarian, breast and kidney cancer) increased risks have been observed in some studies but not all [1]. The outcome of a recent meta-analysis was that acrylamide intake was positively associated with an increased risk of ovarian cancer among never-smoking women (hazard ratio for high versus low intake: 1.39, 95% CI: 0.97–2.00) [1]. On the other hand, a recent study from the EPIC cohort published after the meta-

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analysis did not show an association [2] as did two studies using acrylamide biomarkers to estimate dietary acrylamide exposure instead of food frequency questionnaires [3, 4].

In the most recent risk assessment of acrylamide by the European Food Safety Authority (EFSA) [5], the epidemiological findings on acrylamide and cancer risk are discussed but not incorporated in the actual risk assessment. The most important reasons are the inconsistency in the findings and the fact that the causality of the observed associations between acrylamide intake and cancer risk is unclear. However, the risks observed in humans are considerably higher than predicted from rodent studies [6] and therefore we need to urgently get more clarity on the association between acrylamide intake and ovarian cancer risk and its causality.

In the present study, we aimed to investigate whether genetic make-up modifies the association between acrylamide and ovarian cancer risk, thereby contributing to evidence on acrylamide's mechanism of action and the causality of the observed association in humans. Identification of stronger associations between acrylamide and ovarian cancer in genetically susceptible individuals (e.g., of a certain *CYP2E1* genotype) increases confidence that the observed association between acrylamide intake and ovarian cancer is not due to chance or bias. In addition, choosing genes that are relevant to the biological pathways of the disease can help to tease out disease-causing mechanisms of acrylamide. Finally, acrylamide is part of a mixture of heat-generated compounds or unhealthy diet which impairs the interpretation of acrylamide being the causative agent. Focusing on genes that are rather specific to acrylamide metabolism (e.g., *CYP2E1*) facilitates this interpretation.

We selected SNPs in candidate genes involved in acrylamide metabolism and in mechanisms through which acrylamide is hypothesized to cause cancer: mechanisms involving sex hormones, oxidative stress, and DNA damage caused by glycidamide, acrylamide's genotoxic metabolite [7]. Previously, we investigated the interaction between genetic make-up and acrylamide intake for endometrial cancer risk, and we observed indications for interaction with SNPs in *CYP2E1* and the deletions of *GSTM1* and *GSTT1* [8].

Subjects and methods

Study cohort, cases and follow-up

The Netherlands Cohort Study on diet and cancer started in September 1986 with the inclusion of 62,573 women, 55–69 years of age. Data on dietary habits and other risk

factors were collected by means of a self-administered questionnaire at baseline in 1986. Approximately 75% of the participants sent in toenail clippings, as requested.

Following the case-cohort approach, ovarian cancer cases, detected by annual computerized record linkages to the Netherlands Cancer Registry and the Netherlands Pathology Registry, were enumerated for the entire cohort, while the accumulated person-years for the entire cohort were estimated from a subcohort of 2589 women randomly sampled from the entire cohort at baseline. This study was approved by the review boards of TNO Nutrition and Food Research (Zeist, the Netherlands) and Maastricht University (Maastricht, the Netherlands). Written informed consent was provided by participants by returning the completed questionnaire. Further details on the design and methods of the study are presented elsewhere [9–12].

After 20.3 years of follow-up, Sept. 1986–Dec. 2006, there were 499 microscopically confirmed invasive primary carcinomas of the ovaries ([ICD-O]-3: C56.9). Cases and subcohort members were excluded from analysis if they reported a diagnosis of cancer (except skin cancer) at baseline, their dietary data were incomplete or inconsistent, if they had not sent in toenail clippings, if they had no or inferior (call rate <95%) data on SNPs or if they reported at baseline to have had a unilateral or bilateral ovariectomy (see Fig. 1).

Acrylamide intake assessment

A valid and reproducible food frequency questionnaire with questions on 150 food items was used for estimating dietary habits [11, 12]. Dietary acrylamide intake was estimated from the mean acrylamide level of foods on the Dutch market, and the frequency of consumption and portion size of the foods, as described in detail elsewhere [13].

Selection of genes and SNPs

The selection of genes was broad and focused on genes involved in (1) acrylamide metabolism and (2) the most often hypothesized mechanisms of acrylamide-induced carcinogenesis [7]: (2a) sex hormonal effect (involving sex hormone synthesis/metabolism or sex hormone nuclear receptors), (2b) oxidative stress and (2c) genotoxicity (DNA repair), or (2d) SNPs in genes that otherwise clearly play a role in carcinogenesis. Genes and SNPs of interest were identified from the literature (HugeNavigator and PubMed) and from a personal communication (for SNP rs1280350 in *MGC12965*) with Jos Kleijnans (Dept. of Toxicogenomics, Maastricht University). Genes from category 2a (sex hormonal pathway) were selected based on the KEGG pathway Steroid Hormone Biosynthesis

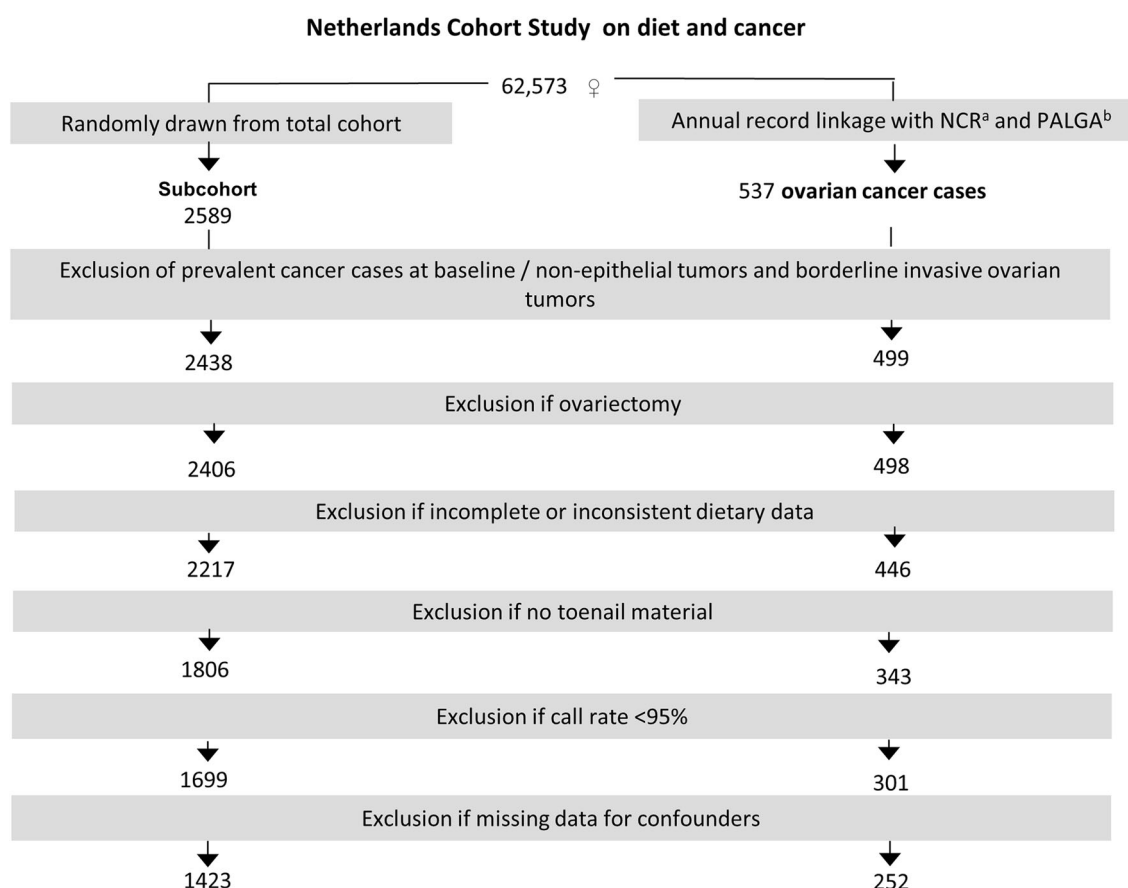


Fig. 1 Flow chart of exclusion steps for ovarian cancer cases and subcohort members

(map00140). Further details on the selection of genes and SNPs were reported elsewhere [8].

In the end, we genotyped 6 SNPs to determine the *GST* deletions and 60 SNPs in other genes, see Supplemental Table 1.

DNA isolation and genotyping

DNA was isolated from 15 mg of toenail clippings, following the protocol developed by Cline et al. [14], in an optimised form [15]. Genotyping was performed by Agena in Hamburg, on the MassARRAY platform using the iPLEX TM assay [16]. This method has been used before to successfully genotype DNA from toenails [8, 15, 17, 18].

Supplemental Table 2 shows the 60 SNPs with their location, call frequencies, and HWE *p* value. 3 out of the 60 SNPs had a call rate <80% and were not included in the analyses. 6 SNPs out of the remaining 57 SNPs did not adhere to Hardy–Weinberg equilibrium (HWE) ($p < 0.05$). With regard to the SNPs selected to represent the *GSTM1* deletion, rs10857795 was not called in 36%, rs200184852 in 42% and rs74837985 in only 2% of the subcohort. The latter value appears to be due to genotyping error. Therefore, we decided to base the assessment of the absence/

presence of the *GSTM1* gene only on rs10857795 and rs200184852. 31% of the subcohort had a missing value for both rs10857795 and rs200184852. With regard to *GSTT1*, rs2844008 was not called in 58%, rs4630 in 16%, and rs140309 in 11% of the subcohort. 8% of the subcohort had a missing value for all 3 *GSTT1* SNPs.

5% of the samples ($n = 190$) were duplicate samples to check the reproducibility of genotyping, which was >99%. We excluded samples with a call rate <95% (42 ovarian cancer cases, 107 subcohort members).

Statistical analysis

Hazard rate ratios (HRs) and 95% confidence intervals were obtained through Cox proportional hazards regression with STATA software (package 13), using the robust Huber–White sandwich estimator to account for additional variance introduced by sampling from the cohort. The proportional hazards assumption was tested using scaled Schoenfeld residuals.

Acrylamide was included in the statistical models as a continuous variable and as quintiles for the main effect of acrylamide and as tertiles in the acrylamide-SNP interaction analyses.

Covariables were selected based on the literature: age, body mass index, height, age at menarche, age at menopause, use of oral contraceptives, parity, use of postmenopausal hormones, and energy intake. Smoking status, the duration of smoking and the number of cigarettes per day were included in the model, because cigarette smoke contains acrylamide [16, 17]. Furthermore, subgroup analyses were performed for never-smokers.

Multiplicative interaction between acrylamide intake and SNPs was tested using product terms of the continuous acrylamide intake variable and genotype. For statistical power reasons, we used a dominant genetic model for all SNPs (i.e., 1 or 2 variant alleles versus homozygous wild type). Tests for acrylamide dose–response trends in genotype strata were performed by fitting the mean acrylamide intake in the tertiles as a continuous variable.

We applied the False Discovery Rate method by Benjamini–Hochberg [19] to adjust for multiple testing with the expected proportion of false positives set at 20%, which is applied regularly in candidate gene studies [20, 21]. We performed separate adjustment for multiple testing for all women and for never-smoking women.

Two-sided *p* values are reported throughout.

Results

Table 1 shows the characteristics of the participants at baseline. Cases were more often never-smokers, and had smoked less and for a shorter duration than subcohort

members. They had less often used oral contraceptives. In addition, cases had fewer children.

Main effect of acrylamide

There was a suggestive (statistically non-significant) positive association between acrylamide and ovarian cancer risk after 20.3 years of follow-up (HR of highest versus the lowest quintile of intake: 1.38 (95% CI 0.95–1.99) and 1.06 (0.98–1.16) per 10 µg/day increment of intake), which was stronger and statistically significant among never-smoking women (HR of highest versus the lowest quintile of intake: 1.85 (95% CI 1.15–2.95) and 1.15 (1.02–1.30) per 10 µg/day increment of intake) (Table 2).

Main effect of the SNPs

Table 3 presents the SNPs showing a clear trend for ovarian cancer over the number of variant alleles. There was an increase in risk with an increasing number of variant alleles for rs511895 in *CAT* (*p* trend = 0.04), rs1056827 in *CYP1B1* (*p* trend = 0.06), and rs2301241 in *TXN* (*p* trend = 0.02). Decreased risks were observed for rs4646903 in *CYP1A1* (*p* = 0.06), rs3219489 in *MUTYH* (*p* trend = 0.05) and the homozygous deletion of *GSTM1* (*p* = 0.03). However, none of the SNPs was statistically significantly associated with ovarian cancer risk after adjustment for multiple comparisons.

Table 1 characteristics of subcohort and ovarian cancer cases

Variable	Ovarian cancer cases	Subcohort
n ^a	364	1474
<i>Dietary variables</i>		
Acrylamide intake (µg/day)	21.9 (13.1)	20.9 (11.8)
Total energy intake (kcal)	1684 (400)	1689 (399)
<i>Non-dietary variables</i>		
Age (yrs)	61.4 (4.3)	61.4 (4.3)
Body mass index (kg/m ²)	25.0 (3.6)	25.1 (3.6)
Age at menarche (yrs)	13.7 (1.8)	13.7 (1.8)
Age at menopause (yrs)	49.0 (4.1)	48.8 (4.4)
Parity, n children	2.4 (2.2)	2.8 (2.2)
n cigarettes per day	3.5 (6.9)	4.5 (7.7)
n smoking years	9.1 (14.5)	11.3 (15.7)
<i>Cigarette smoking status %</i>		
Never smokers	64.8	58.7
Former smokers	19.6	20.9
Current smokers	15.6	20.4
Ever use of postmenopausal hormone treatment, % yes	12.1	13.3
Ever use of oral contraceptives, % yes	16.4	25.4

^a n represents number of subcohort members or cases after exclusion of participants with prevalent cancer at baseline, ovariectomy, incomplete or inconsistent dietary data, and a sample call rate <95%. The number of missing values varies for the variables in this Table

Table 2 Main association between acrylamide intake and ovarian cancer risk, 20.3 years of follow-up

	n cases	Per 10 µg/day increment HR (95% CI) ^a	Quintile 1 HR (95% CI)	Quintile 2 HR (95% CI)	Quintile 3 HR (95% CI)	Quintile 4 HR (95% CI)	Quintile 5 HR (95% CI)	<i>p</i> trend
All women	373	1.06 (0.98–1.16)	Ref (1.00)	1.07 (0.73–1.54)	1.10 (0.75–1.61)	1.05 (0.71–1.53)	1.38 (0.95–1.99)	0.13
Never-smoking women	243	1.15 (1.02–1.30)	Ref (1.00)	1.37 (0.85–2.21)	1.61 (0.98–2.65)	1.50 (0.92–2.44)	1.85 (1.15–2.95)	0.01

Hazard ratios are adjusted for age (years), age at menarche (years), age at menopause (years), parity (n children), ever use of oral contraceptives (yes/no), ever use of postmenopausal hormone treatment (yes/no), height (cm), body mass index (kg/m²), energy intake (kcal/day), and in the analyses for all women: smoking status (never/ex/current smoker), smoking quantity (n cigarettes/day), smoking duration (smoking years)

The median acrylamide intake of the female subcohort in the quintiles was 9.5, 14.0, 17.9, 24.3, and 36.8 µg/day

^a HR (95% CI): hazard ratio with corresponding 95% confidence interval

Table 3 Genetic variants showing a clear dose–response relationship in their association with ovarian cancer risk, 20.3 years of follow-up

Main effects SNPs	Homozygous wildtype		1 or 2 variant alleles		1 variant allele		2 variant alleles		<i>p</i> trend per allele	Benjamini–Hochberg-adjusted <i>p</i> value
	N cases	HR (95% CI) ^a	N cases	HR (95% CI) ^a	N cases	HR (95% CI) ^a	N cases	HR (95% CI) ^a		
<i>CAT</i> , rs511895	86	Ref	215	1.25 (0.95–1.63)	154	1.17 (0.88–1.56)	61	1.48 (1.04–2.13)	0.04	0.59
<i>CYP1A1</i> , rs4646903	261	Ref	36	0.70 (0.48–1.02)	36	0.70 (0.48–1.02)	na		0.06	0.59
<i>CYP1B1</i> , rs1056827	144	Ref	154	1.26 (0.99–1.62)	127	1.24 (0.96–1.61)	27	1.36 (0.87–2.14)	0.06	0.59
<i>MUTYH</i> , rs3219489	189	Ref	112	0.78 (0.60–1.00)	97	0.79 (0.61–1.03)	15	0.70 (0.40–1.23)	0.05	0.59
<i>TXN</i> , rs2301241	95	Ref	206	1.26 (0.97–1.65)	147	1.18 (0.89–1.56)	59	1.55 (1.08–2.22)	0.02	0.59
<i>GSTM1</i> deletion	1 or 2 alleles present		Homozygous deletion		<i>p</i> value		Benjamini–Hochberg-adjusted <i>p</i> value			
	N cases	HR (95% CI) ^a	N cases	HR (95% CI) ^a						
Deletion represented by										
Both <i>GSTM1</i> SNPs	226	Ref	75	0.74 (0.56–0.98)	0.03		0.59			
rs10857795	214	Ref	87	0.73 (0.56–0.95)	0.02		0.59			
rs200184852	185	Ref	116	0.84 (0.66–1.09)	0.19		0.59			

^a HR (95% CI): hazard ratio with corresponding 95% confidence interval; hazard ratios are adjusted for age; *na* not applicable

Interaction between acrylamide and SNPs

None of the SNPs showed a statistically significant multiplicative interaction with acrylamide after adjustment for multiple comparisons. In Table 4, we show interactions with SNPs in genes involved in acrylamide metabolism that are interesting because they have a higher *a priori* probability of modifying the association between acrylamide and cancer risk than the other selected SNPs. Rs915906 and rs2480258 in *CYP2E1* did not show a statistically significant interaction with acrylamide intake among all women (*p* interaction = 0.52 and 0.45,

respectively) nor among never-smoking women (*p* interaction = 0.92 and 0.87, respectively). However, for both SNPs, acrylamide was only positively associated with ovarian cancer risk in women homozygous for the wild type allele and in never-smokers, there was a clear but statistically non-significant dose–response trend for acrylamide for rs915906 (*p* trend = 0.08) and a clear and statistically significant dose–response trend for rs2480258 (*p* trend = 0.04). The homozygous deletion of *GSTM1* did not show an interaction with acrylamide intake but when the deletion was represented by rs4630, acrylamide was only positively associated with ovarian cancer risk in

Table 4 Interactions between SNPs in acrylamide-metabolizing genes and dietary acrylamide intake on the risk of ovarian cancer, 20.3 years of follow-up

SNP ^a	Acrylamide, continuous intake 10 µg/day	Acrylamide, tertiles of intake				Interaction			
		Tertile 1		Tertile 2		Tertile 3		<i>p</i> for trend	
		N cases	HR (95% CI) ^c	N cases	HR (95% CI) ^c	N cases	HR (95% CI) ^c	Raw <i>p</i>	Benjamini–Hochberg adjusted <i>p</i> value
All									
<i>CYP2E1</i> , rs915906 = 0 ^b	1.12 (0.99–1.26)	55	Ref (1.00)	50	0.98 (0.64–1.50)	78	1.35 (0.91–2.01)	0.12	0.52
<i>CYP2E1</i> , rs915906 = 1 ^b	1.00 (0.76–1.32)	33	Ref (1.00)	14	0.42 (0.20–0.87)	22	0.65 (0.33–1.27)	0.21	
Never-smokers									
<i>CYP2E1</i> , rs915906 = 0	1.18 (1.01–1.38)	32	Ref (1.00)	38	1.36 (0.80–2.32)	49	1.57 (0.95–2.59)	0.08	0.96
<i>CYP2E1</i> , rs915906 = 1	1.09 (0.77–1.53)	20	Ref (1.00)	9	0.45 (0.17–1.19)	15	0.72 (0.30–1.72)	0.47	
All									
<i>CYP2E1</i> , rs2480258 = 0	1.13 (0.99–1.28)	51	Ref (1.00)	47	1.03 (0.66–1.62)	73	1.40 (0.93–2.13)	0.10	0.78
<i>CYP2E1</i> , rs2480258 = 1	0.98 (0.79–1.22)	37	Ref (1.00)	17	0.43 (0.22–0.84)	27	0.66 (0.37–1.20)	0.18	
Never-smokers									
<i>CYP2E1</i> , rs2480258 = 0	1.19 (1.02–1.40)	30	Ref (1.00)	36	1.52 (0.87–2.64)	47	1.75 (1.04–2.97)	0.04	0.96
<i>CYP2E1</i> , rs2480258 = 1	1.07 (0.78–1.48)	22	Ref (1.00)	11	0.43 (0.18–1.02)	17	0.59 (0.26–1.34)	0.24	
All									
<i>CYP2E1</i> , rs6413432 = 0	1.07 (0.96–1.19)	71	Ref (1.00)	60	0.94 (0.66–1.34)	85	1.09 (0.79–1.52)	0.58	0.93
<i>CYP2E1</i> , rs6413432 = 1	1.04 (0.74–1.47)	17	Ref (1.00)	4	0.19 (0.06–0.57)	15	0.76 (0.29–1.97)	0.49	
Never-smokers									
<i>CYP2E1</i> , rs6413432 = 0	1.09 (0.94–1.25)	46	Ref (1.00)	44	1.07 (0.70–1.65)	54	1.05 (0.69–1.58)	0.83	0.65
<i>CYP2E1</i> , rs6413432 = 1	1.49 (0.89–2.49)	6	Ref (1.00)	3	0.20 (0.04–1.06)	10	0.92 (0.24–3.49)	0.98	
All									
<i>GSTM1</i> present, all SNPs	1.07 (0.94–1.22)	65	Ref (1.00)	48	0.79 (0.51–1.21)	76	1.09 (0.73–1.61)	0.62	0.90
<i>GSTM1</i> deleted, all SNPs	1.15 (0.90–1.47)	23	Ref (1.00)	16	0.65 (0.31–1.35)	24	1.02 (0.50–2.08)	0.92	
Never-smokers									
<i>GSTM1</i> present, all SNPs	1.13 (0.96–1.32)	40	Ref (1.00)	34	1.04 (0.60–1.79)	47	1.25 (0.76–2.05)	0.37	0.76
<i>GSTM1</i> deleted, all SNPs	1.29 (0.89–1.86)	12	Ref (1.00)	13	1.07 (0.43–2.62)	17	1.25 (0.51–3.03)	0.62	
All									
<i>GSTT1</i> present, rs4630	1.15 (1.03–1.29)	68	Ref (1.00)	52	0.83 (0.56–1.25)	89	1.36 (0.94–1.97)	0.09	0.67
<i>GSTT1</i> deleted, rs4630	0.79 (0.53–1.19)	20	Ref (1.00)	12	0.50 (0.20–1.24)	11	0.31 (0.12–0.77)	0.01	
Never-smokers									
<i>GSTT1</i> present, rs4630	1.23 (1.06–1.44)	40	Ref (1.00)	41	1.14 (0.69–1.87)	57	1.59 (0.99–2.54)	0.05	0.65
<i>GSTT1</i> deleted, rs4630	0.87 (0.53–1.44)	12	Ref (1.00)	6	0.52 (0.15–1.81)	7	0.34 (0.10–1.22)	0.10	

Table 4 continued

SNP ^a	Acrylamide, continuous intake 10 µg/day	Acrylamide, tertiles of intake				Interaction			
		Tertile 1		Tertile 2		Tertile 3		<i>p</i> for trend	
		N cases	HR (95% CI) ^c	N cases	HR (95% CI) ^c	N cases	HR (95% CI) ^c	Raw <i>p</i>	Benjamini–Hochberg adjusted <i>p</i> value
All									
<i>GSTP1</i> , rs1695 = 0	1.05 (0.88–1.25)	31	Ref (1.00)	32	0.96 (0.58–1.58)	38	0.99 (0.59–1.66)	0.81	0.90
<i>GSTP1</i> , rs1695 = 1	1.07 (0.94–1.23)	57	Ref (1.00)	32	0.63 (0.41–0.97)	62	1.02 (0.70–1.50)	0.90	
Never-smokers									
<i>GSTP1</i> , rs1695 = 0	1.07 (0.85–1.36)	19	Ref (1.00)	25	1.17 (0.65–2.11)	24	0.91 (0.48–1.70)	0.79	0.96
<i>GSTP1</i> , rs1695 = 1	1.13 (0.95–1.34)	33	Ref (1.00)	22	0.73 (0.42–1.26)	40	1.09 (0.66–1.79)	0.74	
All									
<i>GSTA5</i> , rs4715354 = 0	0.98 (0.80–1.20)	24	Ref (1.00)	20	1.20 (0.56–2.54)	25	1.06 (0.53–2.13)	0.87	0.81
<i>GSTA5</i> , rs4715354 = 1	1.13 (0.99–1.28)	64	Ref (1.00)	44	0.71 (0.46–1.08)	75	1.15 (0.78–1.69)	0.43	
Never-smokers									
<i>GSTA5</i> , rs4715354 = 0	1.03 (0.80–1.32)	14	Ref (1.00)	13	1.48 (0.55–3.94)	19	1.33 (0.56–3.13)	0.55	0.83
<i>GSTA5</i> , rs4715354 = 1	1.21 (1.00–1.46)	38	Ref (1.00)	34	0.97 (0.58–1.65)	45	1.25 (0.75–2.07)	0.38	
All									
<i>EPHX1</i> , rs1051740 = 0	1.06 (0.89–1.27)	46	Ref (1.00)	26	0.55 (0.34–0.89)	47	0.86 (0.55–1.35)	0.87	0.93
<i>EPHX1</i> , rs1051740 = 1	1.07 (0.94–1.22)	42	Ref (1.00)	38	0.98 (0.62–1.53)	53	1.19 (0.78–1.81)	0.41	
Never-smokers									
<i>EPHX1</i> , rs1051740 = 0	1.10 (0.88–1.38)	31	Ref (1.00)	20	0.63 (0.36–1.12)	31	0.79 (0.46–1.37)	0.41	0.96
<i>EPHX1</i> , rs1051740 = 1	1.12 (0.94–1.33)	21	Ref (1.00)	27	1.32 (0.74–2.36)	33	1.36 (0.77–2.40)	0.30	

Hazard ratios are adjusted for age (years), age at menarche (years), age at menopause (years), parity (n children), ever use of oral contraceptives (yes/no), ever use of postmenopausal hormone treatment (yes/no), height (cm), body mass index (kg/m²), energy intake (kcal/day), and in the analyses for all women: smoking status (never/ex/current smoker), smoking quantity (n cigarettes/day), smoking duration (smoking years)

The median acrylamide intake of the female subcohort in the quintiles was 9.5, 14.0, 17.9, 24.3, and 36.8 µg/day

^a SNP: single nucleotide polymorphism

^b 0: homozygous wildtypes, 1: 1 or 2 variant alleles

^c HR (95% CI): hazard ratio with corresponding 95% confidence interval

women with at least 1 copy of the *GSTT1* gene, with a p for trend of 0.09 among all women and 0.05 among never-smokers. There was no interaction between the deletion of *GSTM1* or other SNPs in acrylamide-metabolizing genes and acrylamide, and no clear difference in the acrylamide-associated risk between the genotypes of these genes.

Supplemental Table 3 shows the results for other SNPs that showed an interaction with acrylamide, or for which the acrylamide-associated risk of ovarian cancer clearly differed between the genotypes. For 5 SNPs in the *HSD3B1/B2* gene cluster, namely rs4659175 (p interaction = 0.04), rs10923823 (p interaction = 0.06) and its proxy rs7546652 (p interaction = 0.05), rs1047303 (p interaction = 0.005), and rs6428830 (p interaction = 0.05), the acrylamide dose-response relationships differed importantly between the genotypes. For all these SNPs, acrylamide intake was only clearly positively associated with ovarian cancer risk among women with 1 or 2 variant alleles. Among never-smoking women, the difference between the genotypes was more pronounced.

Discussion

The current study is the first to analyze acrylamide-gene interactions for ovarian cancer risk. We carefully selected SNPs in genes involved in acrylamide metabolism and genes involved in pathways involved in the mechanism by which acrylamide might cause cancer: a sex hormonal effect, oxidative stress and DNA damage, or otherwise.

CYP2E1

Glycidamide (formed by epoxidation of acrylamide through CYP2E1) is often thought to be the compound responsible for acrylamide-induced carcinogenesis due to genotoxicity. Therefore, studying the modifying effect of SNPs in *CYP2E1* on the association between acrylamide and cancer risk contributes important information on the causality of the association. There was no statistically significant interaction between the 3 studied SNPs in *CYP2E1* and acrylamide intake for ovarian cancer risk. However, similar to endometrial cancer risk [8], where nominally statistically significant interactions were observed for rs915906 and rs2480258, we observed increased acrylamide-associated risks of ovarian cancer only in women homozygous for the wild type allele of both SNPs. As discussed previously [8], this would suggest that acrylamide itself is the causative compound in ovarian carcinogenesis, because the strongest association between acrylamide and ovarian cancer risk was observed among homozygous wild types, suggesting another mechanism of action than genotoxicity. Rs2480258 in *CYP2E1* was not in

Hardy-Weinberg equilibrium, although with a minor deviation ($p = 0.03$). This may indicate that the genotypes for this SNP were measured with some error but there is no reason to assume that this error is different for cases and subcohort members or for different categories of acrylamide intake. Therefore, this potential genotyping error would rather lead to missing a true interactions, if any [22].

GSTs

We observed that women with at least one copy of *GSTT1* were at an increased acrylamide-associated risk of ovarian cancer, which was also what we observed for endometrial cancer [8] but the number of cases with a homozygous deletion of the *GSTT1* gene was very small ($n = 43$). Also similar to endometrial cancer, the homozygous deletion of *GSTM1* was nominally statistically significantly associated with a reduced risk of ovarian cancer, and the homozygous deletion of *GSTT1* was statistically non-significantly associated [among all women: HR: 0.59 (0.18–1.95); never-smokers: HR: 0.58 (0.13–2.55)] with a reduced risk of ovarian cancer. In a recent meta-analysis, there was no association between the null genotypes of *GSTM1* and *GSTT1* and ovarian cancer risk [23]. Unlike for endometrial cancer, there was no difference in the association between acrylamide intake and ovarian cancer risk between the genotypes of *GSTM1*.

A possible explanation for the inverse association between the null genotypes of *GSTM1* and *GSTT1* and ovarian cancer risk is that GSTs catalyze the conjugation of reduced glutathione (GSH) to compounds that protect against ovarian cancer or that they bioactivate compounds involved in ovarian carcinogenesis, for instance catechol estrogens [24]. Conjugation of acrylamide with GSH can result in depletion of cellular GSH stores, leading to altered gene expression directly or through regulating various redox-dependent transcription factors [7]. Considering the fact that acrylamide induces GST activity [25, 26], it would be expected that the positive association between acrylamide and ovarian cancer is only present among women with at least one copy of the genes in whom the activity of GST can be induced.

Hsd3b1/2

We observed nominally statistically significant interaction between acrylamide intake and 5 SNPs in the *HSD3B1/B2* gene cluster of which 2 were complete proxies: rs7546652 and rs10923823 ($R^2 = 1$, $D' = 1$). The 3 β -hydroxysteroid dehydrogenase/ $\delta 5$ -4 is a key rate-limiting enzyme in steroid biosynthesis pathways producing progesterone and androgens. Two studies in mice have shown that acrylamide down-regulated the expression of *HSD3B2*.

(personal communication with Prof. Nan Mei, December 2014 + [25]) Acrylamide has repeatedly been shown to decrease progesterone and testosterone levels in mice and rats [27–29]. Thus, although speculative, the observed interactions between SNPs in the *HSD3B* genes and acrylamide suggest that acrylamide may be involved in ovarian carcinogenesis through effects on progesterone or androgens, since progesterone probably suppresses ovarian carcinogenesis [30–35], and androgens may induce ovarian carcinogenesis [35]. A cross-sectional study on the association between acrylamide intake and progesterone in premenopausal women found no indications for an association between the two but in the same study there were positive associations between acrylamide intake and DHEAS and testosterone in overweight postmenopausal women [36].

Other genes

In addition, for some SNPs, there were no statistically significant indications for interaction but still a clear difference (strongest among never-smokers) in the association between acrylamide intake and ovarian cancer risk between the genotypes: rs11252859 in *AKR1C1* (also involved in progesterone and androgen metabolism), rs3448 in *GPXI*, rs11632903 in *CYP19A1*, rs1800566 in *NQO1*, rs1052133 in *OGG1*, rs824811 and rs8192120 in *SRD5A1* (also involved in progesterone and androgen metabolism), and rs2228000 in *XPC*, rs1056827 in *CYP1B1*, rs2987983 in *ESR2*, rs1280350 in *MGC12965*, rs944722 in *NOS2*, and rs5275 in *PTGS2*. It is, however, premature to elaborately discuss their possible role in acrylamide-induced ovarian carcinogenesis here.

Interactions between SNPs and acrylamide intake for both endometrial [8] and ovarian cancer (this paper) lacked statistical significance after adjustment for multiple testing, probably partly due to a lack of statistical power because in many instances there was a clear difference in the acrylamide-associated risk between genotypes. However, it is worthwhile to look at the overlap between the SNPs for both cancers. The following SNPs showed a nominally statistically significant interaction with acrylamide intake for both endometrial and ovarian cancer, with the same genotypes showing the strongest positive association between acrylamide and cancer risk in never-smokers: rs11252859 in *AKR1C1*, rs3448 in *GPXI*, and rs1800566 in *NQO1*. Additionally, there were clear differences in the acrylamide dose–response between the same genotypes for both cancers for: rs1280350 in *MGC1295* (among never-smokers), and rs6428830 in the *HSD3B1/B2* gene cluster (particularly among never-smokers). These SNPs are worthwhile investigating in future studies on acrylamide intake and endometrial and ovarian cancer risk.

Limitations

This study has some limitations. In the present analysis for ovarian cancer, acrylamide intake was statistically significantly associated with an increased ovarian cancer risk after 20.3 years of follow-up, while the association was only present in the first 11.3 years of follow-up for endometrial cancer [8]. We have no clear explanation for this but it is possible that, due to the fact that endometrial and ovarian cancer are different tumors with a different etiology and partly differing risk factors, acrylamide may have a different role in the etiology of these tumors. An example of the different etiologies of these cancers is that estrogens are thought to play a major role in the etiology of endometrial cancer [37], while they seem to less do so in the etiology of ovarian cancer, which seems to be more clearly influenced by progesterone and androgens [38].

Some of the interactions that we discussed are probably chance findings, considering that none of the SNPs survived adjustment for multiple comparisons. However, finding interactions for multiple SNPs in the *HSD3B1/B2* gene cluster decreases the likelihood that they are chance findings, especially with clear differences in the dose–response pattern of acrylamide between the genotypes.

The statistical power to detect interactions was probably too low for analyses where subgroups based on genotype and acrylamide intake category were small, especially when adjusted for multiple comparisons.

We were unable to assess dietary acrylamide intake with the acrylamide to hemoglobin adduct biomarker because we did not collect blood from the study participants. However, we are not convinced that using biomarkers to estimate acrylamide intake is always necessarily superior to using questionnaires. There are various reasons why acrylamide and glycidamide to hemoglobin adducts (AA and GA Hb-adducts) may not be perfect long-term exposure markers. AA and GA Hb-adducts display large intra-individual variability, as shown by Vikstrom et al. [39], which is probably due to variations in intake of acrylamide-containing foods. This is probably due to intermittent high intakes of foods containing high concentrations of acrylamide which considerably impact the value of the AA and GA Hb-adducts. Similar levels of adducts can arise from a low exposure over an extended time period and from a high incidental exposure. This is not desirable, because for investigating the relationship with cancer, it is probably more important to know the long-term average. Further, acrylamide and glycidamide Hb-adducts are expressed per gram of globin, which means that two persons with the same acrylamide intake may have different AA and GA Hb-adduct levels, dependent on their hemoglobin status. There are many factors that influence hemoglobin levels, such as sex, age, smoking, alcohol intake, physical

exercise, and diet. In addition, the biomarker is not specific for the source of exposure and both active and passive smoking influence AA and GA Hb-adduct levels.

Strengths of this study are the complete follow-up, the prospective nature, and the fact that we observed a main association between acrylamide intake and endometrial and ovarian cancer risk, indicating that acrylamide intake was probably assessed reasonably well in this study.

Conclusion

This study showed nominally statistically significant interactions between several SNPs in the *HSD3B1/B2* gene cluster and acrylamide intake for ovarian cancer risk, suggesting that acrylamide may cause ovarian cancer through effects on sex hormones. Based on this study and our study on endometrial cancer [8], we recommend follow-up of interactions between acrylamide intake and SNPs for ovarian and endometrial cancer risk, particularly SNPs in *CYP2E1*, *GSTs*, the *HSD3B1/B2* gene cluster, *AKR1C1*, *NQO1*, *GPX1* and *MGC12965*.

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Compliance with ethical standards

Conflicts of interest The authors have no conflict of interest to declare. Leo Schouten was compensated for being on an expert panel of the European Food Safety Authority that contributed to the 2015 risk assessment on acrylamide.

Ethical approval This study was approved by the review boards of TNO Nutrition and Food Research (Zeist, the Netherlands) and Maastricht University (Maastricht, the Netherlands). Written informed consent was provided by participants by returning the completed questionnaire. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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References

1. Pelucchi C, Bosetti C, Galeone C, La Vecchia C. Dietary acrylamide and cancer risk: an updated meta-analysis. *Int J Cancer*. 2015;136(12):2912–22. doi:10.1002/ijc.29339.
2. Obon-Santacana M, Peeters PH, Freisling H, Dossus L, Clavel-Chapelon F, Baglietto L, et al. Dietary intake of acrylamide and epithelial ovarian cancer risk in the European prospective investigation into cancer and nutrition (EPIC) cohort. *Cancer Epidemiol Biomark Prev*. 2015;24(1):291–7. doi:10.1158/1055-9965.EPI-14-0636.
3. Xie J, Terry KL, Poole EM, Wilson KM, Rosner BA, Willett WC, et al. Acrylamide hemoglobin adduct levels and ovarian cancer risk: a nested case-control study. *Cancer Epidemiol Biomark Prev*. 2013;22(4):653–60. doi:10.1158/1055-9965.EPI-12-1387.
4. Obon-Santacana M, Lujan-Barroso L, Travis RC, Freisling H, Ferrari P, Severi G, et al. Acrylamide and glycidamide hemoglobin adducts and epithelial ovarian cancer: a nested case-control study in nonsmoking postmenopausal women from the EPIC cohort. *Cancer Epidemiol Biomark Prev*. 2016;25(1):127–34. doi:10.1158/1055-9965.EPI-15-0822.
5. EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). Scientific Opinion on acrylamide in food. *EFSA Journal*. 2015;13(6):4104–321. doi:10.2903/j.efsa.2015.4104.
6. Mucci LA, Adami HO. The role of epidemiology in understanding the relationship between dietary acrylamide and cancer risk in humans. *Adv Exp Med Biol*. 2005;561:39–47.
7. Besaratinia A, Pfeifer GP. A review of mechanisms of acrylamide carcinogenicity. *Carcinogenesis*. 2007;28(3):519–28.
8. Hogervorst JG, van den Brandt PA, Godschalk RW, van Schooten FJ, Schouten LJ. The influence of single nucleotide polymorphisms on the association between dietary acrylamide intake and endometrial cancer risk. *Sci Rep*. 2016;6:34902. doi:10.1038/srep34902.
9. van den Brandt PA, Goldbohm RA, van't Veer P, Volovics A, Hermus RJ, Sturmans F. A large-scale prospective cohort study on diet and cancer in The Netherlands. *J Clin Epidemiol*. 1990;43(3):285–95.
10. van den Brandt PA, Schouten LJ, Goldbohm RA, Dorant E, Hunen PM. Development of a record linkage protocol for use in the Dutch Cancer Registry for Epidemiological Research. *Int J Epidemiol*. 1990;19(3):553–8.
11. Goldbohm RA, van den Brandt PA, Brants HA, van't Veer P, Al M, Sturmans F, et al. Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. *Eur J Clin Nutr*. 1994;48(4):253–65.
12. Goldbohm RA, van't Veer P, van den Brandt PA, van't Hof MA, Brants HA, Sturmans F, et al. Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. *Eur J Clin Nutr*. 1995;49(6):420–9.
13. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomark Prev*. 2007;16(11):2304–13.
14. Cline RE, Laurent NM, Foran DR. The fingernails of Mary Sullivan: developing reliable methods for selectively isolating endogenous and exogenous DNA from evidence. *J Forensic Sci*. 2003;48(2):328–33.
15. Hogervorst JG, Godschalk RW, van den Brandt PA, Weijenberg MP, Verhage BA, Jonkers L, et al. DNA from nails for genetic analyses in large-scale epidemiologic studies. *Cancer Epidemiol Biomark Prev*. 2014;23(12):2703–12. doi:10.1158/1055-9965.EPI-14-0552.
16. Gabriel S, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr Protoc Hum Genet*. 2009. doi:10.1002/0471142905.hg0212s60.

17. Geybels MS, van den Brandt PA, Schouten LJ, van Schooten FJ, van Breda SG, Rayman MP, et al. Selenoprotein gene variants, toenail selenium levels, and risk for advanced prostate cancer. *J Nat Cancer Inst.* 2014;106(3):dju003. doi:[10.1093/jnci/dju003](https://doi.org/10.1093/jnci/dju003).
18. Deckers IA, van den Brandt PA, van Engeland M, van Schooten FJ, Godschalk RW, Keszei AP, et al. Polymorphisms in genes of the renin-angiotensin-aldosterone system and renal cell cancer risk: interplay with hypertension and intakes of sodium, potassium and fluid. *Int J Cancer.* 2015;136(5):1104–16. doi:[10.1002/ijc.29060](https://doi.org/10.1002/ijc.29060).
19. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc.* 1995;57:289–300.
20. Geybels MS, van den Brandt PA, van Schooten FJ, Verhage BA. Oxidative stress-related genetic variants, pro- and antioxidant intake and status, and advanced prostate cancer risk. *Cancer Epidemiol Biomark Prev.* 2015;24(1):178–86. doi:[10.1158/1055-9965.EPI-14-0968](https://doi.org/10.1158/1055-9965.EPI-14-0968).
21. Kim C, Zheng T, Lan Q, Chen Y, Foss F, Chen X, et al. Genetic polymorphisms in oxidative stress pathway genes and modification of BMI and risk of non-Hodgkin lymphoma. *Cancer Epidemiol Biomark Prev.* 2012;21(5):866–8. doi:[10.1158/1055-9965.EPI-12-0010](https://doi.org/10.1158/1055-9965.EPI-12-0010).
22. Fardo DW, Becker KD, Bertram L, Tanzi RE, Lange C. Recovering unused information in genome-wide association studies: the benefit of analyzing SNPs out of Hardy–Weinberg equilibrium. *Eur J Hum Genet.* 2009;17(12):1676–82. doi:[10.1038/ejhg.2009.85](https://doi.org/10.1038/ejhg.2009.85).
23. Han LY, Liu K, Lin XL, Zou BB, Zhao JS. Lack of any association of GST genetic polymorphisms with susceptibility to ovarian cancer—a meta-analysis. *Asian Pac J Cancer Prev.* 2014;15(15):6131–6.
24. Butterworth M, Lau SS, Monks TJ. Formation of catechol estrogen glutathione conjugates and gamma-glutamyl transpeptidase-dependent nephrotoxicity of 17beta-estradiol in the golden Syrian hamster. *Carcinogenesis.* 1997;18(3):561–7.
25. Lee T, Manjanatha MG, Aidoo A, Moland CL, Branham WS, Fuscoe JC, et al. Expression analysis of hepatic mitochondria-related genes in mice exposed to acrylamide and glycidamide. *J Toxicol Environ Health A.* 2012;75(6):324–39. doi:[10.1080/15287394.2012.668160](https://doi.org/10.1080/15287394.2012.668160).
26. Sen A, Ozgun O, Arinc E, Arslan S. Diverse action of acrylamide on cytochrome P450 and glutathione S-transferase isozyme activities, mRNA levels and protein levels in human hepatocarcinoma cells. *Cell Biol Toxicol.* 2012;28(3):175–86. doi:[10.1007/s10565-012-9214-1](https://doi.org/10.1007/s10565-012-9214-1).
27. Wei Q, Li J, Li X, Zhang L, Shi F. Reproductive toxicity in acrylamide-treated female mice. *Reprod Toxicol.* 2014;46:121–8. doi:[10.1016/j.reprotox.2014.03.007](https://doi.org/10.1016/j.reprotox.2014.03.007).
28. Lebda M, Gad S, Gaafar H. Effects of lipoic acid on acrylamide induced testicular damage. *Mater Sociomed.* 2014;26(3):208–12. doi:[10.5455/msm.2014.26.208-212](https://doi.org/10.5455/msm.2014.26.208-212).
29. Shuming C, Jilin F, Xichun Z. The moderating role of dark soy sauce to acrylamide-induced oxidative stress and neurophysiological perturbations in rats. *Toxicol Mech Methods.* 2009;19(6–7):434–40. doi:[10.1080/15376510903136895](https://doi.org/10.1080/15376510903136895).
30. Nagendra PB, Goad J, Nielsen S, Rassam L, Lombard JM, Nahar P, et al. Ovarian hormones through Wnt signalling regulate the growth of human and mouse ovarian cancer initiating lesions. *Oncotarget.* 2017;7:64836–53.
31. Diep CH, Daniel AR, Mauro LJ, Knutson TP, Lange CA. Progesterone action in breast, uterine, and ovarian cancers. *J Mol Endocrinol.* 2015;54(2):R31–53. doi:[10.1530/JME-14-0252](https://doi.org/10.1530/JME-14-0252).
32. Liao J, Ding D, Sun C, Weng D, Meng L, Chen G, et al. Polymorphisms of progesterone receptor and ovarian cancer risk: a systemic review and meta-analysis. *J Obstet Gynaecol Res.* 2015;41(2):178–87. doi:[10.1111/jog.12519](https://doi.org/10.1111/jog.12519).
33. Modugno F. Ovarian cancer and polymorphisms in the androgen and progesterone receptor genes: a HuGE review. *Am J Epidemiol.* 2004;159(4):319–35.
34. Modugno F, Laskey R, Smith AL, Andersen CL, Haluska P, Oesterreich S. Hormone response in ovarian cancer: time to reconsider as a clinical target? *Endocr Relat Cancer.* 2012;19(6):R255–79. doi:[10.1530/ERC-12-0175](https://doi.org/10.1530/ERC-12-0175).
35. Risch HA. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. *J Nat Cancer Inst.* 1998;90(23):1774–86.
36. Hogervorst JG, Fortner RT, Mucci LA, Tworoger SS, Eliassen AH, Hankinson SE, et al. Associations between dietary acrylamide intake and plasma sex hormone levels. *Cancer Epidemiol Biomark Prev.* 2013;22(11):2024–36. doi:[10.1158/1055-9965.EPI-13-0509](https://doi.org/10.1158/1055-9965.EPI-13-0509).
37. Audet-Walsh E, Lepine J, Gregoire J, Plante M, Caron P, Tetu B, et al. Profiling of endogenous estrogens, their precursors, and metabolites in endometrial cancer patients: association with risk and relationship to clinical characteristics. *J Clin Endocrinol Metab.* 2011;96(2):E330–9. doi:[10.1210/jc.2010-2050](https://doi.org/10.1210/jc.2010-2050).
38. Lukanova A, Kaaks R. Endogenous hormones and ovarian cancer: epidemiology and current hypotheses. *Cancer Epidemiol Biomark Prev.* 2005;14(1):98–107.
39. Vikstrom AC, Warholm M, Paulsson B, Axmon A, Wirfalt E, Tornqvist M. Hemoglobin adducts as a measure of variations in exposure to acrylamide in food and comparison to questionnaire data. *Food Chemical Toxicol.* 2012;50(7):2531–9. doi:[10.1016/j.fct.2012.04.004](https://doi.org/10.1016/j.fct.2012.04.004).



Interaction between dietary acrylamide intake and genetic variants for estrogen receptor-positive breast cancer risk

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Abstract

Purpose The association between dietary acrylamide intake and estrogen receptor-positive (ER+) breast cancer risk in epidemiological studies is inconsistent. By analyzing gene–acrylamide interactions for ER+ breast cancer risk, we aimed to clarify the role of acrylamide intake in ER+ breast cancer etiology.

Methods The prospective Netherlands Cohort Study on diet and cancer includes 62,573 women, aged 55–69 years. At baseline, a random subcohort of 2589 women was sampled from the total cohort for a case–cohort analysis approach. Dietary acrylamide intake of subcohort members ($n = 1449$) and ER+ breast cancer cases ($n = 844$) was assessed with a food frequency questionnaire. We genotyped single nucleotide polymorphisms (SNPs) in genes in acrylamide metabolism, sex steroid systems, oxidative stress and DNA repair. Multiplicative interaction between acrylamide intake and SNPs was assessed with Cox proportional hazards analysis, based on 20.3 years of follow-up.

Results Unexpectedly, there was a statistically non-significant inverse association between acrylamide and ER+ breast cancer risk among all women but with no clear dose–response relationship, and no association among never smokers. Among the results for 57 SNPs and 2 gene deletions, rs1056827 in *CYP1B1*, rs2959008 and rs7173655 in *CYP11A1*, the *GSTT1* gene deletion, and rs1052133 in *hOGG1* showed a statistically significant interaction with acrylamide intake for ER+ breast cancer risk.

Conclusions This study did not provide evidence for a positive association between acrylamide intake and ER+ breast cancer risk. If anything, acrylamide was associated with a decreased ER+ breast cancer risk. The interaction with SNPs in *CYP1B1* and *CYP11A1* suggests that acrylamide may influence ER+ breast cancer risk through sex hormone pathways.

Keywords Dietary acrylamide · Single nucleotide polymorphism · Estrogen receptor-positive breast cancer · Prospective cohort

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Introduction

Acrylamide, a probable human carcinogen (IARC class 2A), was discovered in 2002 in various heat-treated carbohydrate-rich foods, such as cookies, potato crisps, French fries and coffee. Acrylamide is a small hydrophilic compound that is distributed throughout the body with the blood. In theory, it can thus cause cancer everywhere in the body. Acrylamide is a multisite carcinogen in rodents, in which it causes, among other, mammary gland tumors in females [1]. The mechanisms by which acrylamide causes mammary gland tumors in rodents are hypothesized to be genotoxicity and endocrine effects [1]. Since 2002, a few epidemiological studies have investigated the impact of dietary acrylamide intake on human cancer risks. The results of these studies

for breast cancer are inconsistent. A recent meta-analysis did not show an increased risk of breast cancer overall, with a relative risk in the highest category of intake versus the lowest of 0.96 (95% CI 0.91–1.02) [2]. However, our previous analysis in the Netherlands Cohort Study [3] and a Danish study using acrylamide hemoglobin adducts as a marker of internal acrylamide exposure [4] gave some indications for a positive association between acrylamide intake and estrogen receptor-positive (ER+) breast cancer risk. Nevertheless, the above-mentioned meta-analysis did not show an increased risk of this type of breast cancer associated with dietary acrylamide intake either [RR 0.98 (95% CI 0.89–1.08)] [2].

In the present study, we investigated whether genetic make-up modifies the association between acrylamide and ER+ breast cancer risk, thereby contributing to evidence on acrylamide's possible mechanism of action and on the causality of the observed associations. We focused on ER+ breast cancer because of the hypothesized effect of acrylamide on sex hormones and the fact that two studies observed an increased acrylamide-associated risk with this subtype of breast cancer. For ER+ breast cancers, the involvement of sex hormones in their etiology is probably stronger than for estrogen receptor-negative breast cancers [5]. We selected SNPs in candidate genes in acrylamide metabolism and in mechanisms through which acrylamide is hypothesized to cause cancer: mechanisms involving sex hormones, oxidative stress, and DNA damage caused by glycidamide, acrylamide's genotoxic metabolite [6]. Previously, we investigated acrylamide intake and gene interactions for endometrial and ovarian cancer risk, and we observed indications for interaction between acrylamide intake and SNPs in among other *cytochrome P450, family 2, subfamily E, polypeptide 1 (CYP2E1)* and the deletions of the genes *glutathione S-transferase M1 and T1 (GSTM1 and GSTT1)* [7, 8].

Subjects and methods

Study cohort, cases and follow-up

The Netherlands Cohort Study on diet and cancer started in September 1986 with the inclusion of 62,573 women that were 55–69 years of age, all presumed to be post-menopausal. Data on dietary habits and other risk factors were collected by means of a self-administered questionnaire at baseline in 1986. In addition to the questionnaire, approximately 75% of the participants sent in toenail clippings, as requested.

Following the case-cohort approach, ER+ breast cancer cases were enumerated for the entire cohort, while the accumulated person-years for the entire cohort were estimated from a subcohort of 2589 women randomly sampled from

the entire cohort at baseline. Since the start of the study, the subcohort has been followed up regularly for vital status information. Incident cancer cases in the total cohort have been detected by computerized record linkages to the Netherlands Cancer Registry, the Netherlands Pathology Registry and the causes of death registry. Further details on the design of the study and methods of follow-up are presented elsewhere [9–12].

After 20.3 years of follow-up, from September 1986 to December 2006, and after exclusion of cohort members who reported a diagnosis of cancer (except skin cancer) at baseline, there were 1620 microscopically confirmed invasive ER+ primary carcinomas of the breast (ICD-O-3: C50). Information on estrogen receptor status was obtained from the National Cancer Registry and the Dutch Pathology Registry and was assessed by either immunohistochemistry or biochemical assay. Cases and subcohort members were excluded from analysis if their dietary data were incomplete or inconsistent, if they had not sent in toenail clippings, and if they had no or inferior (call rate < 95%) data on SNPs. Figure 1 shows the selection and exclusion steps that resulted in the numbers of cases and subcohort members that were available for analysis.

Acrylamide intake assessment

A valid and reproducible food frequency questionnaire with questions on 150 food items was used for estimating dietary habits [11, 12]. Dietary acrylamide intake was estimated from the mean acrylamide level of foods on the Dutch market, and the frequency of consumption and portion size of the foods, as described in detail elsewhere [13].

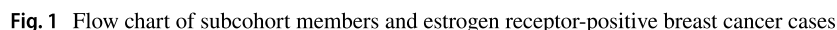
Selection of genes and SNPs

The selection of genes focused on genes involved in (1) acrylamide metabolism and (2) the most often hypothesized mechanisms of acrylamide-induced carcinogenesis [6]: (2a) sex hormonal effect (involving sex hormone synthesis/metabolism or sex hormone nuclear receptors), (2b) oxidative stress and (2c) genotoxicity (DNA repair), or (2d) SNPs in genes that have clearly been shown in the literature to play a role in carcinogenesis. A detailed description of the selection of genes and SNPs is presented elsewhere [7].

In the end, we genotyped 6 SNPs to determine *GSTM1* and *GSTT1* deletions (3 SNPs each) and 60 SNPs in other genes, see Supplemental Table 1.

DNA isolation and genotyping

DNA was isolated from 15 mg of toenail clippings, following the protocol developed by Cline et al. [14], in an optimised form [15]. Genotyping was performed by Agena in



5% of the samples ($n = 190$) were duplicate samples in order to check the reproducibility of genotyping, which was $>99\%$. We excluded samples with a call rate $<95\%$ (113 breast cancer cases, 93 subcohort members).

Covariables selected for inclusion in the Cox proportional hazards analysis models were selected based on the literature: age, body mass index, height, age at menarche, age at menopause, age at first childbirth, parity, ever use of oral contraceptives, ever use of postmenopausal hormones, history of benign breast disease, family history of breast cancer and energy intake. Smoking status, the duration of smoking and the number of cigarettes per day were included in the model, because cigarette smoke contains acrylamide. Smokers have on average four times higher levels of acrylamide-hemoglobin adducts than non-smokers [19, 20]. To eliminate the influence of acrylamide through smoking, we performed subgroup analyses restricted to never smokers. In addition, we checked the confounding potential of various dietary factors, e.g., alcohol, fibre, glycaemic index, but

none changed the hazard ratio of acrylamide by more than 10%. The main associations between SNPs and ER+ breast cancer risk were only adjusted for age.

Multiplicative interaction between acrylamide intake and SNPs was tested using product terms of the continuous acrylamide intake variable and genotype. For statistical power reasons, we used a dominant genetic model for all SNPs. Tests for acrylamide dose–response trends in strata of the genotypes were performed by fitting the mean acrylamide intake in the tertiles as a continuous variable.

We applied the false discovery rate method developed by Benjamini–Hochberg to adjust for multiple testing [21] with the expected proportion of false positives set at 20% [22, 23].

Two-sided *p* values are reported throughout this paper.

Results

Table 1 shows the characteristics of the subcohort and ER+ breast cancer cases at baseline. Cases reported to have fewer children and to more often be nulliparous than subcohort members. Cases reported more often to be current smokers, to have smoked more cigarettes and for a longer duration, and to have drunk more alcohol than subcohort members. They reported more often to have ever used postmenopausal hormone treatment and less often to have ever used oral contraceptives. Furthermore, cases reported more often to have a personal history of benign breast disease and family history of breast cancer.

There was a statistically non-significant inverse association between acrylamide and ER+ breast cancer risk after 20.3 years of follow-up in all women (HR of highest versus the lowest quintile of intake: 0.85 (95% CI 0.66–1.09) and 0.94 (0.88–1.00) per 10 µg/day increment of intake) but with no clear dose–response relationship. There was no association in never-smoking women (HR of highest versus the lowest quintile of intake: 1.18 (95% CI 0.85–1.64) and 1.02 (0.93–1.11) per 10 µg/day increment of intake) (Table 2).

Table 3 presents the SNPs showing a trend for ER+ breast cancer over the number of variant alleles after 20.3 years of follow-up. None of the SNPs was statistically significantly associated with ER+ breast cancer risk after adjustment for multiple comparisons. There were some nominally statistically significant interactions. There was a statistically non-significant decrease in risk with an increasing number of variant alleles for rs2070959 in *UDP glucuronosyltransferase family 1 member A complex (UGT1A)* (*p* trend=0.08), rs4919682 and rs4919687 in *cytochrome P450, family 17, subfamily A, polypeptide 1 (CYP17A1)* (*p* trend=0.07 and 0.05, respectively), rs915906 in *CYP2E1* (*p* trend=0.09), and rs3219489 in *human MutY homolog (hMYH)* (*p* trend=0.07).

Table 1 Characteristics of subcohort and estrogen receptor-positive breast cancer cases

Variable	ER+ breast cancer cases	Subcohort
<i>n</i>	364	1474
Dietary variables		
Acrylamide intake (µg/day)	20.6 (11.3)	21.0 (11.8)
Coffee (g/day)	498 (247)	499 (242)
Dutch spiced cake (g/day)	5.3 (9.0)	5.6 (9.5)
Cookies (g/day)	13.4 (11.5)	13.7 (10.6)
Potato crisps (g/day)	0.36 (1.36)	0.39 (1.80)
French fries (g/day)	3.8 (8.3)	3.7 (8.1)
Alcohol intake (g/day)	6.4 (10.7)	5.9 (9.6)
Total energy intake (kcal)	1689 (394)	1688 (396)
Non-dietary variables		
Age (years)	61.2 (4.2)	61.4 (4.3)
Height (cm)	166 (6)	165 (6)
BMI (kg/m ²)	25.3 (3.3)	25.1 (3.6)
Age at menarche (years)	13.5 (1.8)	13.7 (1.8)
Age at menopause (years)	49.0 (4.5)	48.8 (4.4)
Parity, <i>n</i> children	2.5 (2.0)	2.8 (2.2)
Age at first childbirth		
Nulliparous	20.7	18.1
15–19 years	1.5	2.0
20–24 years	19.3	20.7
25–29 years	38.3	41.2
> 30 years	20.2	18.0
<i>n</i> cigarettes per day	5.0 (8.1)	4.5 (7.7)
<i>n</i> cigarette smoking years	12.0 (16.1)	11.2 (15.6)
Cigarette smoking status %		
Never smokers	56.4	58.8
Former smokers	22.0	21.2
Current smokers	21.6	20.0
Ever use of postmenopausal hormone treatment, % yes	14.5	13.6
Ever use of oral contraceptives, % yes	24.7	25.5
History of benign breast disease, % yes	12.3	7.3
Family history of breast cancer, % yes	14.1	8.4

n represents number of subcohort members or cases after exclusion of participants with prevalent cancer at baseline, incomplete or inconsistent dietary data, and a sample call rate <95%. The number of missing values varies for the variables in this Table

Table 4 shows the interactions between SNPs and acrylamide intake that remained statistically significant after adjustment for multiple comparisons.

The homozygous deletion of *GSTT1*, when represented by rs140309, showed a statistically significant interaction with acrylamide intake (*p* interaction=0.01) and this interaction remained statistically significant after adjustment for multiple testing (Benjamini–Hochberg adjusted *p* value 0.19). The same interaction was observed for never smokers. Women

Table 2 Main association between acrylamide intake and estrogen receptor-positive breast cancer risk, 20.3 years of follow-up

Main effect acrylamide	N cases	HR Per 10 µg/day increment	HR Quintile 1	HR Quintile 2	HR Quintile 3	HR Quintile 4	HR Quintile 5	p trend
All women	1238	0.94 (0.88–1.00)	Ref (1.00)	0.88 (0.69–1.11)	1.01 (0.79–1.29)	0.93 (0.73–1.20)	0.85 (0.66–1.09)	0.37
Never-smoking women	703	1.02 (0.93–1.11)	Ref (1.00)	1.08 (0.78–1.49)	1.44 (1.04–2.01)	1.34 (0.96–1.86)	1.18 (0.85–1.64)	0.17

Hazard ratios (HR) are adjusted for age (years), age at menarche (years), age at menopause (years), age at first childbirth (nulliparous, 15–19 years, 20–24 years, 25–29 years, ≥ 30 years), parity (*n* children), ever use of oral contraceptives (yes/no), ever use of postmenopausal hormone treatment (yes/no), height (cm), body mass index (kg/m²), educational level (4 levels) energy intake (kcal/day), history of benign breast disease, family history of breast cancer, and in the analyses for all women: smoking status (never/ex/current smoker), smoking quantity (*n* cigarettes/day), smoking duration (smoking years)

Table 3 Genetic variants showing a trend for estrogen receptor-positive breast cancer risk, 20.3 years of follow-up

Main effects SNPs	Total N cases	1 or 2 variant alleles versus homozygous wild type		1 variant allele versus homozygous wild type		2 variant alleles versus homozygous wild type		p trend per allele	Benjamini–Hochberg adjusted <i>p</i> value*
		N cases	HR (95% CI) [†]	N cases	HR (95% CI) [†]	N cases	HR (95% CI) [†]		
<i>UGT1A</i> , rs2070959	1040	536	0.87 (0.75–1.02)	450	0.88 (0.75–1.04)	86	0.83 (0.62–1.10)	0.08	0.84
<i>CYP17A1</i> , rs4919682	1039	477	0.82 (0.70–0.95)	392	0.79 (0.67–0.93)	85	0.95 (0.71–1.28)	0.07	0.84
<i>CYP17A1</i> , rs4919687	1040	494	0.82 (0.71–0.96)	407	0.81 (0.69–0.96)	87	0.89 (0.67–1.18)	0.05	0.84
<i>CYP2E1</i> , rs915906	1039	255	0.82 (0.69–0.98)	230	0.80 (0.66–0.96)	25	1.16 (0.68–1.98)	0.09	0.84
<i>hMYH</i> , rs3219489	1039	425	0.91 (0.78–1.06)	378	0.95 (0.81–1.12)	47	0.67 (0.47–0.97)	0.07	0.84

[†]Hazard ratios (HR) are adjusted for age

*Proportion of false positives threshold set at 0.2

with a homozygous deletion of the *GSTT1* gene were at a statistically significantly decreased acrylamide-associated risk of ER+ breast cancer [HR 0.35 (95% CI 0.15–0.83) among all women and HR 0.16 (95% CI 0.04–0.68) among never-smoking women in the highest tertile of acrylamide intake versus the lowest], while there was no association with acrylamide intake among women with at least 1 copy of the gene.

There were four more interactions that remained statistically significant after adjustment for multiple testing, with the following SNPs: rs1056827 in *cytochrome P450, family 1, subfamily B, polypeptide 1 (CYP1B1)* (Benjamini–Hochberg adjusted *p* value 0.18), rs2959008 and rs7173655 (*R*² 0.79, *D'* 0.92) in *cytochrome P450, family 11, subfamily A, polypeptide 1 (CYP11A1)* (Benjamini–Hochberg adjusted *p* value 0.19 for both SNPs), and rs1052133 in *human 8-oxo-7,8-dihydroguanine DNA glycosylase 1 (hOGG1)* (Benjamini–Hochberg adjusted *p* value 0.19). Women homozygous for the wild-type allele of *CYP1B1* were at a statistically significantly decreased acrylamide-associated risk of ER+ breast cancer, while women with at least 1

variant allele were not. Among never smokers, this pattern was not seen, although the interaction was nominally statistically significant (*p* interaction = 0.03). In this subgroup, there was a statistically non-significant increase in risk in women with at least one variant allele, while there was no association between acrylamide and risk in women with two wild-type alleles. The two SNPs in *CYP11A1* both showed that women homozygous for the wild-type allele were at a decreased acrylamide-associated risk while there was no association for women with at least one variant allele. This was seen both among all women and among never smokers. Only women with one or two variant alleles of rs1052133 in *hOGG1* were at a decreased acrylamide-associated risk of ER+ breast cancer. Women who were homozygous wild types did not show an association between acrylamide and ER+ breast cancer. The effect modification was less clear among never smokers.

In Table 5, we show interactions with SNPs in (other) genes involved in acrylamide metabolism that are interesting because they have a higher a priori probability of modifying the association between acrylamide and cancer risk

Table 4 Statistically significant interactions^a between SNPs and dietary acrylamide intake on the risk of estrogen receptor-positive breast cancer, 20.3 years of follow-up

SNP	Acrylamide, continuous intake HR 10 µg/day	Acrylamide, tertiles of intake						Interaction	
		N cases	HR Tertile 1	N cases	HR Tertile 2	N cases	HR Tertile 3	<i>p</i> for linear interaction	
								Raw <i>p</i>	Benjamini–Hochberg adjusted <i>p</i> value [§]
All									
<i>CYP1B1</i> , rs1056827 = 0	0.87 (0.78–0.98)	150	Ref (1.00)	141	0.97 (0.71–1.35)	141	0.83 (0.60–1.15)	0.003	0.18
<i>CYP1B1</i> , rs1056827 = 1	1.05 (0.94–1.18)	134	Ref (1.00)	143	1.08 (0.77–1.50)	132	1.06 (0.75–1.49)		
Never smokers									
<i>CYP1B1</i> , rs1056827 = 0	0.95 (0.81–1.10)	84	Ref (1.00)	78	1.06 (0.68–1.66)	86	0.98 (0.64–1.51)	0.03	0.44
<i>CYP1B1</i> , rs1056827 = 1	1.16 (0.99–1.36)	68	Ref (1.00)	90	1.49 (0.97–2.30)	78	1.35 (0.87–2.10)		
All									
<i>CYP11A1</i> , rs2959008 = 0	0.83 (0.73–0.95)	133	Ref (1.00)	135	1.03 (0.71–1.50)	109	0.73 (0.51–1.06)	0.01	0.19
<i>CYP11A1</i> , rs2959008 = 1	1.04 (0.94–1.16)	153	Ref (1.00)	151	1.05 (0.77–1.42)	164	1.10 (0.81–1.50)		
Never smokers									
<i>CYP11A1</i> , rs2959008 = 0	0.87 (0.74–1.02)	73	Ref (1.00)	77	1.42 (0.86–2.35)	66	0.92 (0.56–1.49)	0.02	0.41
<i>CYP11A1</i> , rs2959008 = 1	1.18 (1.02–1.36)	80	Ref (1.00)	92	1.23 (0.82–1.86)	98	1.35 (0.90–2.03)		
All									
<i>CYP11A1</i> , rs7173655 = 0	0.84 (0.74–0.95)	139	Ref (1.00)	142	1.07 (0.76–1.51)	108	0.71 (0.50–1.02)	0.01	0.19
<i>CYP11A1</i> , rs7173655 = 1	1.03 (0.93–1.14)	146	Ref (1.00)	144	1.02 (0.75–1.40)	165	1.11 (0.81–1.452)		
Never smokers									
<i>CYP11A1</i> , rs7173655 = 0	0.89 (0.75–1.04)	74	Ref (1.00)	80	1.33 (0.83–2.14)	65	0.89 (0.55–1.43)	0.02	0.41
<i>CYP11A1</i> , rs7173655 = 1	1.17 (1.02–1.34)	78	Ref (1.00)	89	1.27 (0.83–1.93)	99	1.43 (0.94–2.17)		
All									
<i>GSTT1</i> present, rs140309	0.97 (0.90–1.06)	252	Ref (1.00)	251	1.02 (0.80–1.30)	255	1.00 (0.78–1.27)	0.01	0.19
<i>GSTT1</i> deleted, rs140309	0.66 (0.48–0.91)	34	Ref (1.00)	35	1.33 (0.55–3.21)	18	0.35 (0.15–0.83)		
Never smokers									
<i>GSTT1</i> present, rs140309	1.08 (0.97–1.21)	132	Ref (1.00)	148	1.26 (0.91–1.76)	152	1.29 (0.93–1.78)	0.01	0.41
<i>GSTT1</i> deleted, rs140309	0.61 (0.37–0.99)	21	Ref (1.00)	21	0.74 (0.21–2.68)	12	0.16 (0.04–0.68)		
All									
<i>hOGG1</i> , rs1052133 = 0	1.03 (0.94–1.14)	152	Ref (1.00)	161	1.08 (0.79–1.46)	182	1.19 (0.89–1.60)	0.02	0.19
<i>hOGG1</i> , rs1052133 = 1	0.81 (0.70–0.93)	134	Ref (1.00)	125	0.91 (0.63–1.31)	91	0.56 (0.37–0.83)		

Table 4 (continued)

SNP	Acrylamide, continuous intake HR 10 µg/day	Acrylamide, tertiles of intake						Interaction	
		<i>N</i> cases	HR Ter- tile 1	<i>N</i> cases	HR Tertile 2	<i>N</i> cases	HR Tertile 3	<i>p</i> for linear interaction	
								Raw <i>p</i>	Benjamini– Hochberg adjusted <i>p</i> value [§]
Never smokers									
<i>hOGG1</i> , rs1052133=0	1.07 (0.95–1.21)	78	Ref (1.00)	92	1.39 (0.93–2.07)	112	1.36 (0.93–2.00)	0.26	0.90
<i>hOGG1</i> , rs1052133=1	0.95 (0.78–1.15)	75	Ref (1.00)	77	1.06 (0.63–1.76)	52	0.81 (0.48–1.38)		

Hazard ratios (HR) are adjusted for age (years), age at menarche (years), age at menopause (years), age at first childbirth (nulliparous, 15–19 years, 20–24 years, 25–29 years, ≥ 30 years), parity (*n* children), ever use of oral contraceptives (yes/no), ever use of postmenopausal hormone treatment (yes/no), height (cm), body mass index (kg/m²), educational level (4 levels) energy intake (kcal/day), history of benign breast disease, family history of breast cancer, and in the analyses for all women: smoking status (never/ex/current smoker), smoking quantity (*n* cigarettes/day), smoking duration (smoking years)

[§]Proportion of false positives threshold set at 0.2

^aAfter adjustment for multiple comparisons

than the other selected SNPs. Rs915906, rs2480258, and rs6413432 in *CYP2E1* did not show an interaction with acrylamide intake among all women, nor among never-smoking women. There was also no interaction between the deletion of *GSTM1* and acrylamide, or SNPs in other acrylamide-metabolizing genes.

Supplemental Table 3 shows the nominally statistically significant interactions that did not withstand adjustment for multiple testing, namely: rs1800566 in *NQO1* and rs6838248 in *SLC7A11* specifically among never smokers.

Finally, there were some clear differences in the association with acrylamide between genotypes without a statistically significant interaction, namely for: rs2070959 in *UGT1A*, rs11252859 in *AKR1C1*, rs11252887 in *AKR1C2*, rs1280350 in *MGC12965*, rs1042157 and rs6839 in *SULT1A1*, rs737865 in *COMT*, rs10432782 in *SOD1*, rs4880 and rs5746136 in *SOD2*, rs1047303 in the *HSD3B1/2* gene cluster, rs6259 in *SHBG*, rs6759180 in *RRM2*, and rs2228001 in *XPC* (Supplemental Table 3).

Discussion

As far as we know, this is the first study to analyze acrylamide-gene interactions for breast cancer risk. We observed interactions between acrylamide intake and rs1056827 in *CYP1B1*, rs2959008 and rs7173655 in *CYP11A1*, the *GSTT1* gene deletion, and rs1052133 in *hOGGI*. These interactions remained statistically significant after adjustment for multiple testing.

Contrary to what we found in a previous analysis for ER+ breast cancer albeit not statistically significant [3],

acrylamide intake was not positively associated with ER+ breast cancer risk among never smokers in the current analysis. In addition, when we restricted our analyses to 13.3 years of follow-up (as in the previous analysis), acrylamide intake was not positively associated with ER+ breast cancer risk. The explanation for this discrepancy is probably that different case sets were used in the analyses. In the previous analyses, cases were derived from the Dutch Pathology Registry and four regional Dutch cancer registries because only those 4 routinely recorded information on estrogen receptor status at that time. Cases for the current analysis originated from all nine regional Dutch cancer registries, the Dutch Pathology Registry and the causes of death registry, and so there were more cases in the present analysis. In the previous analysis, the percentage of cases with missing info on estrogen receptor status was quite high (57%) and we checked whether cases with known estrogen receptor status differed from cases with unknown receptor status with regard to tumor and other characteristics, such as BMI and age. This was not the case but it is still possible that selection of a specific subgroup of ER+ cases occurred and that the positive association between acrylamide intake and ER+ breast cancer risk was restricted to this group. Among all women in the current analysis, there was a tendency towards an inverse association.

Glycidamide (the epoxide metabolite of acrylamide formed through metabolism by *CYP2E1*) is often thought to be responsible for acrylamide-induced carcinogenesis due to genotoxicity (mutagenicity and/or clastogenicity [24]). Studying the modifying effect of SNPs in *CYP2E1* on the association between acrylamide and cancer risk may thus contribute important information on the causality of the

Table 5 Interactions between SNPs in acrylamide-metabolizing genes and dietary acrylamide intake on the risk of estrogen receptor-positive breast cancer, 20.3 years of follow-up

SNP	Acrylamide, continuous intake		Acrylamide, tertiles of intake				Interaction	
	HR 10 µg/day	N cases	Acrylamide, tertiles of intake		N cases	HR Tertile 3	p for trend	p for linear interaction
			HR Tertile 1	N cases				
All								
<i>CYP2E1</i> , rs915906 = 0	0.93 (0.85–1.02)	212	Ref (1.00)	219	1.03 (0.79–1.35)	0.87 (0.67–1.14)	0.30	0.84
<i>CYP2E1</i> , rs915906 = 1	0.95 (0.79–1.15)	74	Ref (1.00)	67	1.03 (0.64–1.65)	0.96 (0.58–1.58)	0.86	
Never smokers								
<i>CYP2E1</i> , rs915906 = 0	1.02 (0.91–1.14)	111	Ref (1.00)	132	1.30 (0.91–1.85)	1.10 (0.77–1.55)	0.67	0.94
<i>CYP2E1</i> , rs915906 = 1	1.07 (0.82–1.39)	42	Ref (1.00)	37	1.19 (0.59–2.40)	1.24 (0.62–2.48)	0.55	0.97
All								
<i>CYP2E1</i> , rs2480258 = 0	0.91 (0.82–1.01)	188	Ref (1.00)	185	0.99 (0.74–1.33)	0.83 (0.62–1.12)	0.21	0.48
<i>CYP2E1</i> , rs2480258 = 1	1.00 (0.87–1.14)	98	Ref (1.00)	101	1.09 (0.74–1.60)	1.02 (0.69–1.51)	0.93	0.83
Never smokers								
<i>CYP2E1</i> , rs2480258 = 0	1.01 (0.89–1.14)	96	Ref (1.00)	110	1.29 (0.87–1.91)	1.12 (0.77–1.63)	0.60	0.68
<i>CYP2E1</i> , rs2480258 = 1	1.05 (0.86–1.28)	57	Ref (1.00)	59	1.11 (0.65–1.88)	1.06 (0.63–1.78)	0.83	0.95
All								
<i>CYP2E1</i> , rs6413432 = 0	0.92 (0.84–1.00)	236	Ref (1.00)	229	1.02 (0.79–1.32)	0.85 (0.66–1.09)	0.19	0.27
<i>CYP2E1</i> , rs6413432 = 1	1.05 (0.85–1.30)	50	Ref (1.00)	57	1.04 (0.58–1.85)	1.13 (0.60–2.11)	0.70	0.70
Never smokers								
<i>CYP2E1</i> , rs6413432 = 0	1.01 (0.91–1.13)	123	Ref (1.00)	136	1.33 (0.93–1.88)	1.13 (0.80–1.58)	0.53	0.40
<i>CYP2E1</i> , rs6413432 = 1	1.15 (0.80–1.66)	30	Ref (1.00)	33	0.87 (0.41–1.84)	1.10 (0.48–2.53)	0.81	0.90
All								
<i>GSTM1</i> present, all SNPs	0.95 (0.86–1.05)	193	Ref (1.00)	198	1.05 (0.79–1.39)	0.86 (0.65–1.15)	0.29	0.82
<i>GSTM1</i> deleted, all SNPs	0.92 (0.79–1.06)	93	Ref (1.00)	88	1.02 (0.66–1.56)	1.06 (0.68–1.66)	0.79	0.90
Never smokers								
<i>GSTM1</i> present, all SNPs	1.06 (0.94–1.20)	93	Ref (1.00)	111	1.55 (1.04–2.30)	1.26 (0.86–1.84)	0.27	0.22
<i>GSTM1</i> deleted, all SNPs	0.92 (0.74–1.13)	60	Ref (1.00)	58	1.00 (0.57–1.77)	1.00 (0.56–1.77)	1.00	0.86
All								
<i>GSTP1</i> , rs1695 = 0	0.93 (0.82–1.06)	123	Ref (1.00)	116	0.86 (0.60–1.24)	0.80 (0.55–1.15)	0.22	0.82
<i>GSTP1</i> , rs1695 = 1	0.95 (0.86–1.05)	163	Ref (1.00)	170	1.16 (0.86–1.57)	0.98 (0.72–1.33)	0.88	0.90
Never smokers								
<i>GSTP1</i> , rs1695 = 0	1.05 (0.87–1.26)	68	Ref (1.00)	74	1.23 (0.74–2.03)	1.00 (0.61–1.63)	0.96	0.74
<i>GSTP1</i> , rs1695 = 1	1.03 (0.90–1.16)	85	Ref (1.00)	95	1.33 (0.88–1.99)	1.22 (0.82–1.83)	0.35	0.95
All								
<i>GSTA5</i> , rs4715354 = 0	0.99 (0.83–1.17)	91	Ref (1.00)	67	0.77 (0.49–1.21)	0.80 (0.51–1.26)	0.34	0.53
<i>GSTA5</i> , rs4715354 = 1								0.83

Table 5 (continued)

SNP	Acrylamide, continuous intake HR 10 µg/day	Acrylamide, tertiles of intake			Interaction		
		N cases			p for trend		
		N cases	HR Tertile 1	N cases	HR Tertile 2	N cases	HR Tertile 3
<i>GSTA5</i> , rs4715354 = 1	0.92 (0.84–1.01)	195	Ref (1.00)	217	1.14 (0.87–1.49)	198	0.95 (0.72–1.24)
Never smokers							
<i>GSTA5</i> , rs4715354 = 0	1.04 (0.85–1.27)	46	Ref (1.00)	43	1.11 (0.59–2.10)	47	1.09 (0.61–1.94)
<i>GSTA5</i> , rs4715354 = 1	1.02 (0.90–1.15)	107	Ref (1.00)	126	1.26 (0.88–1.81)	117	1.10 (0.76–1.58)
All							
<i>EPHX1</i> , rs1051740 = 0	0.96 (0.84–1.09)	152	Ref (1.00)	133	0.74 (0.53–1.03)	131	0.70 (0.49–0.98)
<i>EPHX1</i> , rs1051740 = 1	0.93 (0.84–1.03)	134	Ref (1.00)	153	1.36 (0.99–1.87)	142	1.15 (0.84–1.58)
Never smokers							
<i>EPHX1</i> , rs1051740 = 0	1.01 (0.86–1.19)	82	Ref (1.00)	88	0.95 (0.61–1.47)	81	0.83 (0.53–1.30)
<i>EPHX1</i> , rs1051740 = 1	1.01 (0.88–1.17)	71	Ref (1.00)	81	1.48 (0.95–2.32)	83	1.32 (0.85–2.05)

Hazard ratios (HR) are adjusted for age (years), age at menarche (years), age at first childbirth (nulliparous, 15–19 years, 20–24 years, 25–29 years, ≥ 30 years), parity (*n* children), ever use of oral contraceptives (yes/no), ever use of postmenopausal hormone treatment (yes/no), height (cm), body mass index (kg/m²), educational level (4 levels) energy intake (kcal/day), history of benign breast disease, family history of breast cancer, and in the analyses for all women: smoking status (never/ex/current smoker), smoking quantity (*n* cigarettes/day), smoking duration (smoking years)

§Proportion of false positives threshold set at 0.2

association. We observed no interaction between 3 SNPs in *CYP2E1* and acrylamide intake for ER+ breast cancer risk and there were no clear differences in the risk between the genotypes, contrary to what was observed for endometrial [7] and ovarian cancer [8]. We have no clear explanation for this inconsistency but an explanation could be that there is no association between acrylamide intake and breast cancer risk or that for breast cancer, acrylamide and glycidamide are (roughly) equally responsible for the carcinogenic effect. The three studied *CYP2E1* SNPs are in the intronic region of the gene and there is no clear information about their functionality but the wild-type allele of rs6413432 has been shown to be associated with an increased risk of prostate cancer [25] and other cancers such as lung cancer [26].

We observed that women with a homozygous deletion of *GSTT1* (deletion represented by rs140309) were at a decreased acrylamide-associated risk of ER+ breast cancer but the number of cases with a homozygous *GSTT1* deletion was rather small ($n = 87$). In contrast, we previously observed women with at least one copy of the *GSTT1* gene to be at an increased acrylamide-associated risk of endometrial and ovarian cancer [7, 8]. There was no clear difference in the association between acrylamide intake and ER+ breast cancer risk between the genotypes of *GSTM1*.

We observed a statistically significant interaction between acrylamide intake and rs1056827 in *CYP1B1*. Women who were homozygous wild types for this allele were at a decreased risk of acrylamide-associated ER+ breast cancer. In never smokers, the interaction was less clear. *CYP1B1* is a phase I biotransformation enzyme involved in the metabolism of various exogenous and endogenous compounds and is mainly expressed in endocrine tissues like the endometrium, ovary and breast. *CYP1B1* converts estrogens to hydroxy metabolites (catechol estrogens) which are potent estrogens and furthermore *CYP1B1* oxidizes catechol estrogens to chemically reactive semiquinone and quinone intermediates that can bind to DNA and cause mutations. Exposure of mouse spermatocytes to acrylamide and glycidamide led to increased *CYP1B1* expression [27], while glycidamide exposure led to decreased *CYP1B1* expression in human epithelial cells [28]. Possible explanations for this discrepancy are species, cell or dose differences. The variant allele of rs1056827 has been shown to have increased enzyme activity and to be associated with an increased breast cancer risk [29]. Due to the scarcity of literature and the inconsistency in the relationship between acrylamide and *CYP1B1* activity, it is currently impossible to say whether the observed interaction is biologically plausible.

Acrylamide interacted statistically significantly with 2 SNPs in *CYP11A1*: rs2959008 and rs7173655; acrylamide intake was associated with a decreased ER+ breast cancer risk in women who were homozygous for the wild type of these alleles. *CYP11A1* is involved in the formation of

pregnenolone from cholesterol, the first and rate-limiting step in steroid hormone synthesis. Both *CYP11A1* SNPs are in the intronic region of the gene and there is no information available about their functionality. The variant allele of rs2959008 was associated with a decreased breast cancer risk in Han Chinese [30]. There is no literature on rs7173655 and breast cancer risk but the variant allele was associated with an increased endometrial cancer risk [31]. Acrylamide exposure of male Fischer 344 rats led to increased expression of *CYP11A1* in reproductive tissues [32] but to decreased *CYP11A1* expression in testis tissue of male Sprague–Dawley rats [33]. This discrepancy may be due to differences in rat strains or doses of acrylamide, or both. Thus, again due to the scarcity and inconsistency of data, it is not currently possible to judge the biological plausibility of the interaction between acrylamide and these *CYP11A1* SNPs.

Progesterone opposes the proliferative effect of estrogens in the endometrium and ovaries while it is thought to have proliferative effects in mammary tissue [34]. A mechanism by which acrylamide may increase risks of endometrial and ovarian cancer and decrease the risk of ER+ breast cancer is through an effect on progesterone. However, this is highly speculative, also due to the fact that we did not see interaction between acrylamide and the *CYP2E1* SNPs which leaves the possibility that there may not be a true association between acrylamide intake and breast cancer risk. If true, the interactions between acrylamide and *HSD3B* SNPs for ovarian cancer [8] and between acrylamide and the *CYP1B1* and *CYP11A1* SNPs for breast cancer in the current study give some indications that acrylamide may interfere with progesterone metabolism. However, there was no association between acrylamide intake and progesterone in a cross-sectional study in premenopausal women [35]. In animals, acrylamide has repeatedly been shown to decrease progesterone levels [36–38]. Nevertheless, we strongly encourage more research on the possible effect of acrylamide on progesterone metabolism in humans.

Only women with variant alleles of rs1052133 in *hOGG1* showed an inverse association between acrylamide intake and ER+ breast cancer risk. The *hOGG1* gene is part of the base excision DNA repair pathway, responsible for the excision of 8-oxoguanine (8-oxoG), a mutagenic DNA base byproduct of reactive oxygen species. Although the variant allele is hypothesized to have decreased enzyme activity [39, 40], a recent meta-analysis showed that it is associated with a decreased risk of breast cancer among Europeans [41], while another meta-analysis did not show an association [42]. Because of these apparent contradictions, it is currently impossible to speculate about the possible mechanism by which this SNP could modify the association between acrylamide intake and ER+ breast cancer risk.

There were two other nominally statistically significant interactions between acrylamide intake and other SNPs:

rs1800566 in *NQO1* and rs6838248 in *SLC7A11*. Additionally, there were some clear differences in the association with acrylamide between genotypes without a statistically significant interaction, for: rs2070959 in *UGT1A*, rs11252859 in *AKR1C1*, rs11252887 in *AKR1C2*, rs1280350 in *MGC12965*, rs1042157 and rs6839 in *SULT1A1*, rs737865 in *COMT*, rs10432782 in *SOD1*, rs4880 and rs5746136 in *SOD2*, rs1047303 in the *HSD3B1/B2* gene cluster, rs6259 in *SHBG*, rs6759180 in *RRM2*, and rs2228001 in *XPC*. For all these SNPs it is even more important that the interaction between acrylamide intake and these SNPs is corroborated in other studies to be able to judge whether our findings represent true interactions or not.

This study has some limitations. Acrylamide levels vary considerably within foods due to processing and varieties used. Despite the large variation in acrylamide levels within foods, acrylamide intake as assessed by food frequency questionnaires and acrylamide to hemoglobin adducts (biomarker for exposure) have been shown to correlate moderately in several studies, e.g. [43, 44]. Thus, the food frequency questionnaire is able to estimate the rank order of acrylamide intake among study populations. In addition, we correlated the assessed acrylamide intake (based on mean acrylamide levels per food) and the measured acrylamide content of 24-hour Dutch duplicate diets, for which the participants had written down exactly what and how much they ate and drank. The correlation was very high ($r=0.82$, $p<0.001$) [45]. To conclude, assessing acrylamide intake through food frequency questionnaires is not perfect and entails some random measurement error, which pushes the point estimate towards the null, but it is useful for studying the link between acrylamide intake and cancer risk. Data on diet and covariables obtained from the questionnaire were collected only once, at baseline. Some of the characteristics (e.g., diet, BMI) will certainly have changed over time after baseline. One of the reasons to select an elderly population for the study was that older people tend to have more stable dietary habits. The changes that have occurred despite this will have resulted in random measurement error, pushing the point estimate of the hazard ratio towards the null.

Some of the nominally statistically significant interactions that we observed are, without a doubt, chance findings. However, it is of interest that some of the genes that we observed to interact with acrylamide for ER+ breast cancer risk or that showed clear differences in risk between the genotypes also did so for endometrial [7] and ovarian cancer [8]: *GSTT1*, *AKR1C1*, *NQO1*, the *HSD3B1/B2* gene cluster, *XPC*, and *MGC12965*. These genes therefore deserve attention in future studies.

The strengths of this study are the complete follow-up and its prospective nature.

In conclusion, we did not observe a positive association between dietary acrylamide intake and ER+ breast cancer

risk. Unexpectedly, our results gave some indications for an inverse association. Unlike for endometrial and ovarian cancer, there was no interaction between acrylamide intake and *CYP2E1* SNPs for ER+ breast cancer risk. After adjustment for multiple testing, this study showed statistically significant interactions between rs1056827 in *CYP1B1*, rs2959008 and rs7173655 in *CYP11A1*, the deletion of *GSTT1*, and rs1052133 in *hOGG1* and acrylamide intake for ER+ breast cancer risk. Based on this study and analyses for endometrial and ovarian cancer, we recommend follow-up of interactions between acrylamide intake and genetic polymorphisms in *CYP1B1*, *CYP11A1*, the *HSD3B1/B2* gene cluster, *CYP2E1*, *GSTs*, *hOGG1*, *AKR1C1*, *NQO1*, *GPX1*, *XPC* and *MGC12965*, and additional research on the possible effect of acrylamide on progesterone metabolism in humans.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare. Leo Schouten was compensated for being on an expert panel of the European Food Safety Authority that contributed to the 2015 risk assessment on acrylamide.

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References

1. Maier A, Kohrman-Vincent M, Hertzberg R, Allen B, Haber LT, Dourson M (2012) Critical review of dose-response options for F344 rat mammary tumors for acrylamide - additional insights based on mode of action. *Food Chem Toxicol* 50(5):1763–1775
2. Pelucchi C, Bosetti C, Galeone C, La Vecchia C (2015) Dietary acrylamide and cancer risk: an updated meta-analysis. *Int J Cancer* 136(12):2912–2922
3. Pedersen GS, Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2010) Dietary acrylamide intake and estrogen and progesterone receptor-defined postmenopausal breast cancer risk. *Breast Cancer Res Treat* 122(1):199–210

4. Olesen PT, Olsen A, Frandsen H, Frederiksen K, Overvad K, Tjønneland A (2008) Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study. *Int J Cancer* 122(9):2094–2100
5. Althuis MD, Fergenbaum JH, Garcia-Closas M, Brinton LA, Madigan MP, Sherman ME (2004) Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. *Cancer Epidemiol Biomarkers Prev* 13(10):1558–1568
6. Besaratinia A, Pfeifer GP (2007) A review of mechanisms of acrylamide carcinogenicity. *Carcinogenesis* 28(3):519–528
7. Hogervorst JG, van den Brandt PA, Godschalk RW, van Schooten FJ, Schouten LJ (2016) The influence of single nucleotide polymorphisms on the association between dietary acrylamide intake and endometrial cancer risk. *Sci Rep* 6:34902
8. Hogervorst JG, van den Brandt PA, Godschalk RW, van Schooten FJ, Schouten LJ (2017) Interactions between dietary acrylamide intake and genes for ovarian cancer risk. *Eur J Epidemiol* 32(5):431–441
9. van den Brandt PA, Goldbohm RA, van 't Veer P, Volovics A, Hermus RJ, Sturmans F (1990) A large-scale prospective cohort study on diet and cancer in The Netherlands. *J Clin Epidemiol* 43(3):285–295
10. van den Brandt PA, Schouten LJ, Goldbohm RA, Dorant E, Hunen PM (1990) Development of a record linkage protocol for use in the Dutch Cancer Registry for Epidemiological Research. *Int J Epidemiol* 19(3):553–558
11. Goldbohm RA, van den Brandt PA, Brants HA, van't Veer P, Al M, Sturmans F, Hermus RJ (1994) Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. *Eur J Clin Nutr* 48(4):253–265
12. Goldbohm RA, van 't Veer P, van den Brandt PA, van 't Hof MA, Brants HA, Sturmans F, Hermus RJ (1995) Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. *Eur J Clin Nutr* 49(6):420–429
13. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2007) A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev* 16(11):2304–2313
14. Cline RE, Laurent NM, Foran DR (2003) The fingernails of Mary Sullivan: developing reliable methods for selectively isolating endogenous and exogenous DNA from evidence. *J Forensic Sci* 48(2):328–333
15. Hogervorst JG, Godschalk RW, van den Brandt PA, Weijenberg MP, Verhage BA, Jonkers L, Goessens J, Simons CC, Vermeesch JR, van Schooten FJ et al (2014) DNA from nails for genetic analyses in large-scale epidemiologic studies. *Cancer Epidemiol Biomarkers Prev* 23(12):2703–2712
16. Gabriel S, Ziaugra L, Tabbaa D (2009) SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr Protoc Hum Genet* Chap 2:Unit 2 12
17. Geybels MS, van den Brandt PA, Schouten LJ, van Schooten FJ, van Breda SG, Rayman MP, Green FR, Verhage BA (2014) Selenoprotein gene variants, toenail selenium levels, and risk for advanced prostate cancer. *J Nat Cancer Inst* 106(3):dju003
18. Deckers IA, van den Brandt PA, van Engeland M, van Schooten FJ, Godschalk RW, Keszei AP, Schouten LJ (2015) Polymorphisms in genes of the renin-angiotensin-aldosterone system and renal cell cancer risk: Interplay with hypertension and intakes of sodium, potassium and fluid. *Int J Cancer* 136(5):1104–1116
19. Schettgen T, Rossbach B, Kutting B, Letzel S, Drexler H, Angerer J (2004) Determination of haemoglobin adducts of acrylamide and glycidamide in smoking and non-smoking persons of the general population. *Int J Hyg Environ Health* 207(6):531–539
20. Bergmark E (1997) Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and nonsmokers. *Chem Res Toxicol* 10(1):78–84
21. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Statist Soc* 57:289–300
22. Geybels MS, van den Brandt PA, van Schooten FJ, Verhage BA (2015) Oxidative stress-related genetic variants, pro- and antioxidant intake and status, and advanced prostate cancer risk. *Cancer Epidemiol Biomarkers Prev* 24(1):178–186
23. Kim C, Zheng T, Lan Q, Chen Y, Foss F, Chen X, Holford T, Leaderer B, Boyle P, Chanock SJ et al (2012) Genetic polymorphisms in oxidative stress pathway genes and modification of BMI and risk of non-Hodgkin lymphoma. *Cancer Epidemiol Biomarkers Prev* 21(5):866–868
24. Wang RS, McDaniel LP, Manjanatha MG, Shelton SD, Doerge DR, Mei N (2010) Mutagenicity of acrylamide and glycidamide in the testes of big blue mice. *Toxicol Sci* 117(1):72–80
25. Ferreira PM, Medeiros R, Vasconcelos A, Costa S, Pinto D, Morais A, Oliveira J, Lopes C (2003) Association between CYP2E1 polymorphisms and susceptibility to prostate cancer. *Eur J Cancer Prev* 12(3):205–211
26. Su XL, Bin B, Cui HW, Ran MR (2011) Cytochrome P450 2E1 Rsal/PstI and DraI polymorphisms are risk factors for lung cancer in mongolian and han population in inner Mongolia. *Chin J Cancer Res* 23(2):107–111
27. Nixon BJ, Katen AL, Stanger SJ, Schjenken JE, Nixon B, Roman SD (2014) Mouse spermatocytes express CYP2E1 and respond to acrylamide exposure. *PLoS one* 9(5):e94904
28. Clement FC, Dip R, Naegeli H (2007) Expression profile of human cells in culture exposed to glycidamide, a reactive metabolite of the heat-induced food carcinogen acrylamide. *Toxicology* 240(1–2):111–124
29. Reding KW, Weiss NS, Chen C, Li CI, Carlson CS, Wilkerson HW, Farin FM, Thummel KE, Daling JR, Malone KE (2009) Genetic polymorphisms in the catechol estrogen metabolism pathway and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 18(5):1461–1467
30. Sun M, Yang X, Ye C, Xu W, Yao G, Chen J, Li M (2012) Risk-association of CYP11A1 polymorphisms and breast cancer among Han Chinese women in Southern China. *Int J Mol Sci* 13(4):4896–4905
31. Terry K, McGrath M, Lee IM, Buring J, De Vivo I (2010) Genetic variation in CYP11A1 and StAR in relation to endometrial cancer risk. *Gynecol Oncol* 117(2):255–259
32. Camacho L, Latendresse JR, Muskhelishvili L, Patton R, Bowyer JF, Thomas M, Doerge DR (2012) Effects of acrylamide exposure on serum hormones, gene expression, cell proliferation, and histopathology in male reproductive tissues of Fischer 344 rats. *Toxicol Lett* 211(2):135–143
33. Yang HJ, Lee SH, Jin Y, Choi JH, Han DU, Chae C, Lee MH, Han CH (2005) Toxicological effects of acrylamide on rat testicular gene expression profile. *Reprod Toxicol* 19(4):527–534
34. Diep CH, Daniel AR, Mauro LJ, Knutson TP, Lange CA (2015) Progesterone action in breast, uterine, and ovarian cancers. *J Mol Endocrinol* 54(2):R31–53
35. Hogervorst JG, Fortner RT, Mucci LA, Tworoger SS, Eliassen AH, Hankinson SE, Wilson KM (2013) Associations between dietary acrylamide intake and plasma sex hormone levels. *Cancer Epidemiol Biomarkers Prev* 22(11):2024–2036
36. Lebda M, Gad S, Gaafar H (2014) Effects of lipoic acid on acrylamide induced testicular damage. *Mater Sociomed* 26(3):208–212
37. Shuming C, Jilin F, Xichun Z (2009) The moderating role of dark soy sauce to acrylamide-induced oxidative stress and neurophysiological perturbations in rats. *Toxicol Mech Methods* 19(6–7):434–440

38. Wei Q, Li J, Li X, Zhang L, Shi F (2014) Reproductive toxicity in acrylamide-treated female mice. *Reprod Toxicol* 46:121–128
39. Kohno T, Shinmura K, Tosaka M, Tani M, Kim SR, Sugimura H, Nohmi T, Kasai H, Yokota J (1998) Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. *Oncogene* 16(25):3219–3225
40. Lee AJ, Hodges NJ, Chipman JK (2005) Interindividual variability in response to sodium dichromate-induced oxidative DNA damage: role of the Ser326Cys polymorphism in the DNA-repair protein of 8-oxo-7,8-dihydro-2'-deoxyguanosine DNA glycosylase 1. *Cancer Epidemiol Biomarkers Prev* 14(2):497–505
41. Yuan Y, Chen F, Zhao GH, Liu J, Zhang HX, Hu XS (2007) A comparative study of acrylamide formation induced by microwave and conventional heating methods. *J Food Sci* 72(4):C212–216
42. Gu D, Wang M, Zhang Z, Chen J (2010) Lack of association between the hOGG1 Ser326Cys polymorphism and breast cancer risk: evidence from 11 case-control studies. *Breast Cancer Res Treat* 122(2):527–531
43. Wilson KM, Bälter K, Adami HO, Grönberg H, Vikström AC, Paulsson B, Törnqvist M, Mucci LA (2009) Acrylamide exposure measured by food frequency questionnaire and hemoglobin adduct levels and prostate cancer risk in the Cancer of the Prostate in Sweden Study. *Int J Cancer* 124(10):2384–2390
44. Wilson KM, Vesper HW, Tocco P, Sampson L, Rosén J, Hellenäs KE, Törnqvist M, Willett WC (2009) Validation of a food frequency questionnaire measurement of dietary acrylamide intake using hemoglobin adducts of acrylamide and glycidamide. *Cancer Causes Control* 20(3):269–278
45. Konings EJ, Hogervorst JG, van Rooij L, Schouten LJ, Sizoo EA, van Egmond HP, Goldbohm RA, van den Brandt PA (2010) Validation of a database on acrylamide for use in epidemiological studies. *Eur J Clin Nutr* 64(5):534–540



Dietary acrylamide intake and risk of endometrial cancer in prospective cohort studies

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Abstract

Purpose Acrylamide has been associated with carcinogenicity in experimental animals, but potential health risks of dietary acrylamide intake and endometrial cancer in human are inconclusive. Thus, a meta-analysis of prospective cohort studies was conducted to provide a quantitative assessment of the association between dietary acrylamide intake and endometrial cancer risk.

Methods PubMed database was used to identify prospective cohort studies on dietary acrylamide intake and endometrial cancer risk published up to June 2014. Since smoking is an important source of acrylamide and is inversely associated with endometrial cancer risk, the association was examined in women who never smoked as well. Multivariable relative risks (RR) adjusting for potential confounders were combined using random effects models.

Results Four large prospective cohort studies were identified, which included 453,355 female participants and 2,019 endometrial cancer cases. There was no association between dietary acrylamide intake and endometrial cancer risk overall [pooled RR for high vs. low intake = 1.10; 95 % confidence interval (CI) 0.91–1.34]. High acrylamide intake, however, was significantly associated with increased risk of endometrial cancer among women who never smoked (pooled RR for high vs. low intake = 1.39; 95 % CI 1.09–1.77). In dose–response analyses, pooled RRs for an increase of 10 µg/day were 1.04 (95 % CI

0.97–1.11) among all women and 1.11 (95 % CI 1.04–1.19) among never-smoking women.

Conclusions Endometrial cancer risk was not associated with dietary acrylamide intake overall. Among women who never smoked, however, there was a significantly increased endometrial cancer risk in women who consumed high dietary acrylamide.

Keywords Acrylamide · Endometrial cancer · Prospective cohort studies · Meta-analysis

Introduction

In 1994, acrylamide was evaluated by the International Agency for Research on Cancer (IARC) as a ‘probable human carcinogen (class 2A)’, based on positive evidence from animal studies and inadequate epidemiologic evidence [1]. In addition that the exposure to acrylamide primarily occurs in occupational setting and through tobacco smoke, it is also found in common human foods (e.g., potato crisps, fried potato, French fries, cookies, coffee, etc.) that are mainly formed during high-temperature cooking as part of the Maillard browning reactions [2, 3]. Some animal studies showed positive dose–response relations between acrylamide administered in drinking water and several types of cancers, especially in hormone-sensitive organs including uterus [4, 5]. Endometrial cancer, especially most prevalent type-I endometrial cancer associated with unopposed estrogen exposure, has been associated with some dietary factors such as coffee consumption, dietary glycemic load, and alcohol drinking [6–9], suggesting that dietary modification may lower endometrial cancer risk to some extent. In a meta-analysis of two prospective cohort studies, no significant association

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between dietary acrylamide intake and endometrial cancer risk was reported [10]. However, it is possible that residual confounding by smoking exists. Therefore, a comprehensive meta-analysis of prospective cohort studies was conducted to provide a quantitative assessment of the association between dietary acrylamide intake and endometrial cancer risk in all women and never-smoking women, separately.

Methods

Search strategy and inclusion criteria

PubMed database was used to identify eligible epidemiologic studies published in English through June 2014. The following terms were used in searching: “(acrylamide, diet, dietary factors or risk factors)” combined with “(corpus uterian or endometrial neoplasms).” The reference lists of original and review articles were also reviewed to identify additional eligible studies. Studies were eligible for inclusion if they met the following criteria: (1) a prospective cohort study design; (2) the exposure of interest was dietary acrylamide intake; (3) the outcome of interest was clearly defined as endometrial cancer (preferentially type-I endometrial cancer) incidence; and (4) adjusted relative risks (RRs) with 95 % confidence intervals (CIs). Authors were directly contacted to ask for the full papers when studies were not available [11].

Data extraction

The following data were extracted according to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) [12]: first author’s last name, year of publication, country name, cohort name, study period, number of cases, number of subjects and person-years, dietary acrylamide intake (quintiles or quartiles), adjustment factors, and multivariable-adjusted RRs with 95 % CIs across categories of acrylamide intake.

Statistical analysis

To combine the RRs from individual studies for high versus low categories of dietary acrylamide intake, random effects models that incorporate both within- and between-study variations were used [13]. The study-specific RRs and pooled RR were presented as a forest plot where the size of data markers (squares) corresponds to the inverse of the variance of the natural logarithm of RR from each study, and the diamond shows pooled RR. Statistical heterogeneity among studies was evaluated using the Cochran’s Q statistic [14], and inconsistency was quantified

with the I^2 (*I*-squared) statistic [15]. To examine a dose-response relationship and calculate a pooled RR for an increase of 10 µg/day of dietary acrylamide intake, a generalized least-squares trend (GLST) estimation analysis was used [16, 17]. Subgroup analyses by geographic regions (Europe/USA) and menopausal status were conducted with tests of whether RRs varied by the characteristics using a random effects meta-regression model. A sensitivity analysis was performed by eliminating one study at a time to assess whether the results were driven by a single study. All of the analyses were repeated in never smokers to eliminate the influence of smoking. Finally, publication bias was evaluated through funnel plots (i.e., a plot of study results against precision) and with Begg’s and Egger’s tests [18, 19]. A two-tailed *p* value of <0.05 was considered statistically significant. All statistical meta-analyses were carried out using Stata/SE version 12.0 software (Stata Corporation, College Station, Texas).

Results

Four large prospective cohort studies that met the inclusion criteria were identified, which included 453,355 female participants and 2,019 endometrial cancer cases [11, 20–22]. The study characteristics included in the meta-analysis are summarized in Table 1. Of the four cohort studies (the Netherlands Cohort Study, NLCS; the Nurses’ Health Study, NHS; the Swedish Mammography Cohort, SMC; and the European Prospective Investigation into Cancer and Nutrition, EPIC cohort), three studies were conducted in the Europe (364,683 women; 1,535 cases) [11, 20, 21], and one study was conducted in the United States (88,672 women; 484 cases) [22]. The NLCS included postmenopausal women only, and the other three studies included both premenopausal and postmenopausal women, which provided separate RRs by menopausal status. Dietary acrylamide intakes of cohort subjects were assessed with food frequency questionnaires (FFQs). For the assessment of dietary acrylamide intake, the NHS used seven FFQs, while the remaining studies used one or two FFQs. In the NHS, cumulative average acrylamide intake was calculated using repeated measurements to best represent long-term dietary exposure to acrylamide for individuals. The four cohort studies also provided data for women who never smoked (1,030 cases). Two cohort studies provided risk estimates of dietary acrylamide intake for type-I endometrial cancer, specifically [11, 22], while the others provided data for overall endometrial cancer only [20, 21]. All studies included in the meta-analysis adjusted for potential confounders such as age, smoking, and body mass index (BMI). Three studies also adjusted for physical activity [20–22].

Table 1 Characteristics of prospective cohort studies included in the meta-analysis of dietary acrylamide intake and endometrial cancer risk

References	Country (cohort)	Study period	Outcome	No. of cases/subjects (or person-years)	High vs. low(ref) acrylamide intake (µg/day)	Relative risk (95 % CI)	Adjustment factors
Hogervorst et al. [20]	Netherland (NLCS)	1986–1997	Overall EC	All 221/2,344 women (15,836) Never smokers 150/NA (9,422)	Q5 (median 36.8) vs. Q1 (9.5)	1.29 (0.81–2.07) 1.99 (1.12–3.52)	Age, age at menarche, age at menopause, age at first childbirth, parity, duration of OC use, duration of PMH use, BMI, height, current smoking, quantity of smoking, duration of smoking, non-occupational physical activity, energy intake, trans-unsaturated fatty acid intake, carbohydrate intake, alcohol consumption
Larsson et al. [21]	Sweden (SMC)	1987–2007	Overall EC	All 687/61,226 women (1,080,747) Never smokers 273/NA (344,580)	Q4 (≥28.9) vs. Q1 (<19.9) Q4 (≥29.2) vs. Q1 (<20.5)	0.96 (0.76–1.21) 1.20 (0.76–1.90)	Age, education, BMI, parity, age at first birth, age at menarche, age at menopause, use of OC, use of PMH, history of diabetes, smoking status, total physical activity, energy-adjusted carbohydrate intake, and total energy intake
Wilson et al. [22]	USA (NHS)	1980–2006	Type-I EC	All 484/88,672 women (1,386,886) Never smokers 257/NA (627,668)	Q5 (mean 26) vs. Q1 (9)	1.41 (1.01–1.97) 1.43 (0.90–2.28)	Age, smoking, BMI, menopausal status/age at menopause/PMH use, parity, OC use, high blood pressure, diabetes, physical activity, caffeine intake, energy intake
Obón-Santacann et al. [11]	Ten European countries (EPIC)	1992–2010	Type-I EC	All 627/301,113 women (3,303,26) Never smokers 350/166,853	Q5 (32.1–222.4) vs. Q1 (≤14.5)	0.97 (0.69–1.36) 1.25 (0.79–1.98)	Age, center, smoking status, OC use, HRT use, total energy intake, BMI, prevalent diabetes, menopause status combined with age at menopause, parity and age at menarche

BMI body mass index (kg/m²), EC endometrial cancer, EPIC European Prospective Investigation into Cancer and Nutrition, HRT hormone replacement therapy, NA not applicable, NHS Nurses' Health Study, NLCS Netherlands Cohort Study, PMH postmenopausal hormone, OC oral contraceptive, SMC Swedish Mammography Cohort

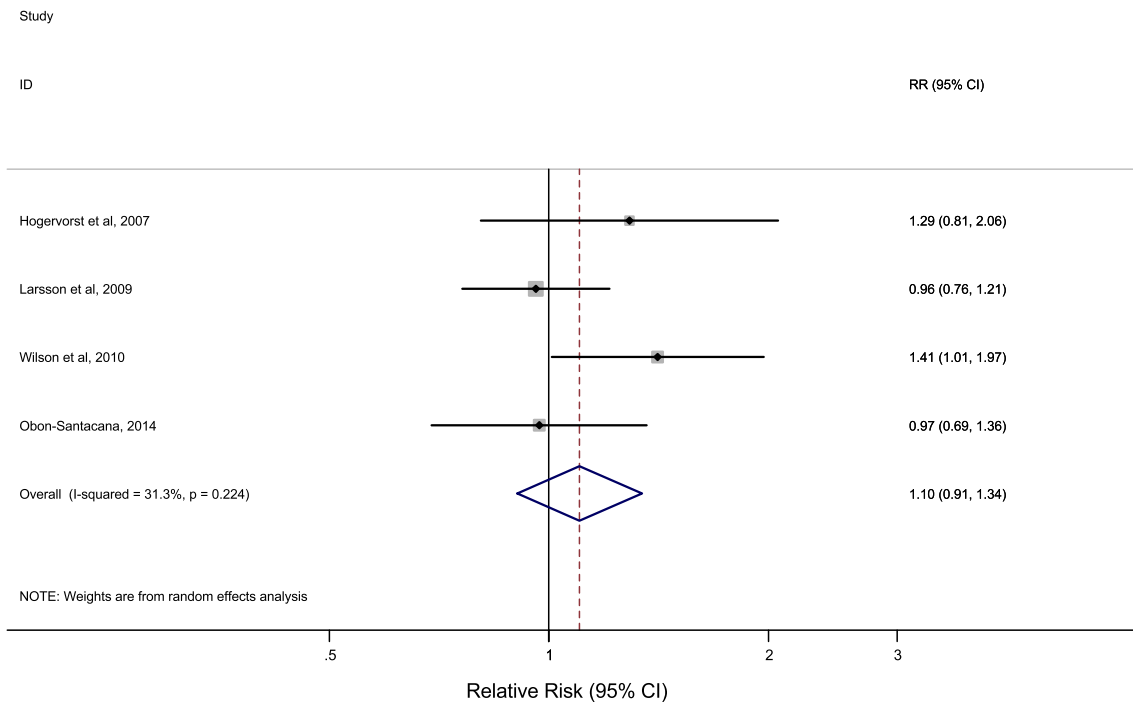


Fig. 1 Study-specific and pooled adjusted relative risks of endometrial cancer for high vs. low categories of dietary acrylamide intake. *CI* confidence interval, *RR* relative risk

Meta-analysis with all subjects

The pooled adjusted RR comparing high (~ 35.2 $\mu\text{g/day}$, mean) vs. low (~ 10.9 $\mu\text{g/day}$, mean) categories of dietary acrylamide intake was 1.10 (95 % CI 0.91–1.34) with no significant heterogeneity among the studies (p for heterogeneity = 0.22, $I^2 = 31.3$ %) (Fig. 1). The pooled RR from three European studies for high vs. low acrylamide intake was 1.00 (95 % CI 0.84–1.20), and there was no significant difference in RRs by country (p for Europe vs. the United States = 0.22). By menopausal status, postmenopausal women had a pooled RR of 1.15 (95 % CI 0.92–1.43), and premenopausal women had a pooled RR of 1.07 (95 % CI 0.53–1.15), and the difference in RRs by menopausal status was not statistically significant ($p = 0.77$). The pooled RRs in the sensitivity analysis that eliminated one study at a time did not differ substantially, all of which included a pooled RR of 1.0 in the confidence intervals, indicating that the observed null association was not driven by one single study. The dose–response analysis also showed no association overall (pooled RR for 10 $\mu\text{g/day}$ = 1.04; 95 % CI 0.97–1.11).

Meta-analysis with women who never smoked

Among never-smoking women, a significantly increased endometrial cancer risk was found in women consuming high dietary acrylamide. The pooled adjusted RR for high

vs. low categories of dietary acrylamide intake was 1.39 (95 % CI 1.09–1.77) with no significant heterogeneity (p for heterogeneity = 0.55, $I^2 = 0.0$ %) (Fig. 2). No significant difference in RRs of studies conducted in Europe and the United States was found ($p = 0.91$). Based on the results of sensitivity analysis among never smokers, pooled RRs did not differ substantially, ranging from 1.29 to 1.47 (95 % CI 1.11–1.96). In the dose–response analysis, the pooled RR for an increase of 10 $\mu\text{g/day}$ of dietary acrylamide intake was 1.11 (95 % CI 1.04–1.19) in never-smoking women.

Publication bias

There was no significant evidence of publication bias in the meta-analysis with all subjects (Begg's $p = 0.73$; Egger's $p = 0.37$) or women who never smoked (Begg's $p = 0.09$; Egger's $p = 0.053$).

Discussion

In this meta-analysis of prospective cohort studies, no significant association between dietary acrylamide intake and endometrial cancer risk was found among all female subjects. However, among women who never smoked, a significantly increased endometrial cancer risk was found in women who consumed high dietary acrylamide. There

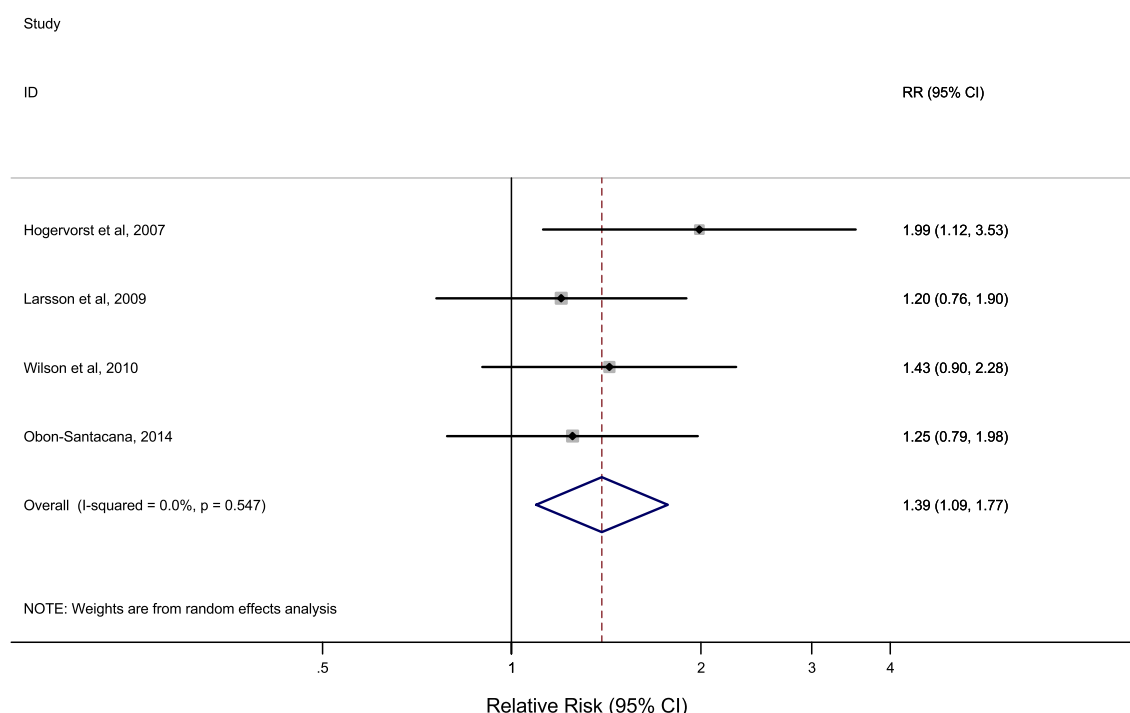


Fig. 2 Study-specific and pooled adjusted relative risks of endometrial cancer for high vs. low categories of dietary acrylamide intake among women who never smoked. *CI* confidence interval, *RR* relative risk

were neither evidences of significant heterogeneity among the studies nor publication bias.

All of the cohort studies included in the meta-analysis tended to show positive associations among women who never smoked, but only one cohort study showed a significant positive association [20]. When the large prospective data were combined through the meta-analytic technique, among women who never smoked, there was a significantly increased endometrial cancer risk in women who consumed high dietary acrylamide by 39 %. An increment of 10 μg of dietary acrylamide intake daily was associated with 11 % increased endometrial cancer risk among women who never smoked. The positive association was not detected when all subjects were included.

Cigarette smoke, an important source of acrylamide exposure, has been associated with lower endometrial cancer risk [23], and thus can act as a negative confounder for the association between dietary acrylamide intake and endometrial cancer risk if unadjusted. All studies included in the meta-analysis adjusted for smoking status in the multivariable models, but residual confounding by smoking remains possible. As a way to remove possible remaining confounding by smoking, a pooled RR of acrylamide intake was calculated in women who never smoked, and there was a significantly increased risk in women who consumed high acrylamide.

The positive association between dietary acrylamide intake and endometrial cancer risk found in never-smoking women is biologically plausible. Acrylamide is metabolized by the CYP2E1 enzyme system to glycidamide, which is a chemically reactive epoxide and mutagen in animals, and there is some evidence of carcinogenicity of acrylamide in experimental animals [4, 5, 24]. In addition, acrylamide may also be carcinogenic through hormonal pathways. Prolonged exposure to excessive estrogens, unopposed by progesterone, has been considered as a major risk factor, resulting in continued stimulation of the endometrium. It is possible that dietary acrylamide may affect activities of enzymes involved in the metabolism of sex steroid hormones and thus have effects on sex hormones [25].

To the best of my knowledge, this is the first meta-analysis of prospective cohort studies to examine dietary acrylamide intake and endometrial cancer risk among women who never smoked, specifically. All of the studies included in the meta-analysis had prospective designs, which had no methodological biases such as recall bias or selection bias found in retrospective studies, and had relatively long follow-up periods. The overall sample size of this meta-analysis was relatively large, and all the cohorts provided separate RRs among all women as well as never-smoking women. Although each study attempted to adjust

for important cofounders in the multivariable models, we cannot rule out the possibility that some unknown or residual confounders may have affected the results given the observational nature of cohort studies. Out of four studies, two studies assessed dietary acrylamide intake, repeatedly [21, 22]. Since the other two studies, however, assessed the dietary acrylamide intake at baseline only [11, 20], the non-differential exposure misclassification may exist, which can attenuate a true relationship between dietary acrylamide intake and endometrial cancer risk.

In conclusion, results from the meta-analysis of prospective cohort studies indicate that there was no association between dietary acrylamide intake and endometrial cancer risk overall. Among women who never smoked, however, there was a significantly increased endometrial cancer risk with increasing acrylamide intake. Further prospective studies with repeated measurements of dietary acrylamide intake, careful adjustment for all potential confounders, and subgroup analyses are needed to examine the association of dietary acrylamide intake and endometrial cancer risk. In addition, the association in never smokers could be evaluated in large consortia (data pooling) of studies, and that acrylamide or glycidamide hemoglobin adducts in blood might also help to verify the possible association observed in never-smoking women.

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Conflict of interest The author declares that I have no conflict of interest.

Ethical standards The manuscript does not contain clinical studies or patient data.

References

1. International Agency for Research on Cancer (IARC) (1994) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Some industrial chemicals, vol. 60 Lyon, France
2. Stadler RH, Blank I, Varga N, Robert F, Hau J, Guy PA, Robert MC, Riediker S (2002) Acrylamide from Maillard reaction products. *Nature* 419:449–450
3. Lipworth L, Sonderman JS, Taronea RE, McLaughlin JK (2012) Review of epidemiologic studies of dietary acrylamide intake and the risk of cancer. *Eur J Cancer Prev* 21:375–386
4. Johnson KA, Gorzinski SJ, Bodner KM, Campbell RA, Wolf CH, Friedman MA, Mast RW (1986) Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol Appl Pharmacol* 85:154–168
5. Friedman MA, Dulak LH, Stedham MA (1995) A lifetime oncogenicity study in rats with acrylamide. *Fundam Appl Toxicol* 27:95–105
6. Je Y, Hankinson SE, Tworoger SS, DeVivo I, Giovannucci E (2011) A prospective cohort study of coffee consumption and risk

- of endometrial cancer over a 26-year follow-up. *Cancer Epidemiol Biomarkers Prev* 20:2487–2495
7. Je Y, Giovannucci E (2012) Coffee consumption and risk of endometrial cancer: findings from a large up-to-date meta-analysis. *Int J Cancer* 131:1700–1710
8. Galeone C, Augustin LS, Filomeno M, Malerba S, Zucchetto A, Pelucchi C, Montella M, Talamini R, Franceschi S, La Vecchia C (2013) Dietary glycemic index, glycemic load, and the risk of endometrial cancer: a case-control study and meta-analysis. *Eur J Cancer Prev* 22:38–45
9. Je Y, DeVivo I, Giovannucci E (2014) Long-term alcohol intake and risk of endometrial cancer in the nurses' health study. *Br J Cancer* 111:186–194
10. Pelucchi CC, La Vecchia C, Bosetti C, Boyle P, Boffetta P (2011) Exposure to acrylamide and human cancer—a review and meta-analysis of epidemiologic studies. *Ann Oncol* 22:1487–1499
11. Obón-Santacana M, Kaaks R, Slimani N, Lujan-Barroso L, Freisling H, Ferrari P, Dossus L, Chabbert-Buffet N, Baglietto L, Fortner RT, Boeing H, Tjønneland A, Olsen A, Overvad K, Menéndez V, Molina-Montes E, Larrañaga N, Chirlaque MD, Ardanaz E, Khaw KT, Wareham N, Travis RC, Lu Y, Merritt MA, Trichopoulou A, Benetou V, Trichopoulos D, Saieva C, Sieri S, Tumino R, Sacerdote C, Galasso R, Bueno-de-Mesquita HB, Wirfält E, Ericson U, Idahl A, Ohlson N, Skeie G, Gram IT, Weiderpass E, Onland-Moret NC, Riboli E, Duell EJ (2014) Dietary intake of acrylamide and endometrial cancer risk in the European Prospective Investigation into cancer and nutrition cohort. *Br J Cancer* 111:987–997
12. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB (2000) Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 283:2008–2012
13. DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. *Control Clin Trials* 7:177–188
14. Cochran WG (1954) The combination of estimates from different experiments. *Biometrics* 10:101–129
15. Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. *BMJ* 327:557–560
16. Greenland S, Longnecker MP (1992) Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol* 135:1301–1309
17. Orsini N, Bellocco R, Greenland S (2006) Generalized least squares for trend estimation of summarized dose-response data. *Stata J* 6:40–57
18. Begg CB, Mazumdar M (1994) Operating characteristics of a rank correlation test for publication bias. *Biometrics* 50:1088–1101
19. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in metaanalysis detected by a simple, graphical test. *BMJ* 315:629–663
20. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2007) A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev* 16:2304–2313
21. Larsson SC, Hakansson N, Akesson A, Wolk A (2009) Long-term dietary acrylamide intake and risk of endometrial cancer in a prospective cohort of Swedish women. *Int J Cancer* 124:1196–1199
22. Wilson KM, Mucci LA, Rosner BA, Willett WC (2010) A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 19:2503–2515
23. Zhou B, Yang L, Sun Q, Cong R, Gu H, Tang N, Zhu H, Wang B (2008) Cigarette smoking and the risk of endometrial cancer: a meta-analysis. *Am J Med* 121:501–508

24. Hogervorst JG, Baars BJ, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2010) The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. *Crit Rev Toxicol* 40:485–512
25. Hogervorst JG, Fortner RT, Mucci LA, Tworoger SS, Eliassen AH, Hankinson SE, Wilson KM (2013) Associations between dietary acrylamide intake and plasma sex hormone levels. *Cancer Epidemiol Biomarkers Prev* 22:2024–2036

ORIGINAL ARTICLE

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Dietary acrylamide intake and risk of breast cancer: The Japan Public Health Center-based Prospective Study

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Acrylamide forms during cooking and is classified as a probable carcinogen in humans, mandating the need for epidemiological studies of dietary acrylamide and cancers. However, the risk of dietary acrylamide exposure to breast cancer in Japanese women has not been assessed. We investigated the association between dietary acrylamide intake and risk of breast cancer in the Japan Public Health Center-based Prospective Study. The present study included 48 910 women aged 45–74 years who responded to a 5-year follow-up survey questionnaire. Dietary acrylamide intake was assessed using a validated food frequency questionnaire. Cox proportional hazards regression models were used to estimate hazard ratios and 95% confidence intervals. During an average of 15.4 years of follow up, 792 breast cancers were diagnosed. Energy-adjusted dietary acrylamide intake was not associated with the risk of breast cancer (adjusted hazard ratio for highest versus lowest tertile = .95, 95% confidence intervals: 0.79–1.14, *P*-trend = .58). Further, no significant associations were observed when stratified analyses were conducted by smoking status, coffee consumption, alcohol consumption, body mass index, menopausal status, estrogen receptor status, and progesterone receptor status. In conclusion, dietary acrylamide intake was not associated with the risk of breast cancer in this population-based prospective cohort study of Japanese women.

KEYWORDS

acrylamide, Asia, breast cancer, diet, epidemiology

1 | INTRODUCTION

Acrylamide was classified as a probable human carcinogen (group 2A) by The International Agency for Research on Cancer in

1994.¹ Until 2002, the main sources of acrylamide exposure were thought to be through specific occupations or smoking.² However, Swedish researchers found that acrylamide occurs in carbohydrate-rich foods cooked at over 120°C, showing that one of the most common forms of acrylamide exposure in the population was from meals.³

The carcinogenicity of dietary acrylamide is considered to occur through a genotoxic pathway.⁴ Acrylamide is soluble in water, absorbed from the gastrointestinal tract, and transported to several organs.⁵ Acrylamide is metabolized by 2 pathways,⁵ a direct pathway by glutathione conjugation of acrylamide by GST, and a second by

Abbreviations: BMI, body mass index; CI, confidence interval; DCO, death certificate only; DR, dietary record; ER, estrogen receptor; FFQ, food frequency questionnaire; HR, hazard ratio; ICD-O-3, International Classification of Diseases for Oncology, Third Edition; JPHC Study, the Japan Public Health Center-based Prospective Study; MOE, margin of exposure; PR, progesterone receptor.

[†]Members of the JPHC Study Group are listed at the following site (as of April 2016): <http://epi.ncc.go.jp/en/jphc/781/3838.html>

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glycidamide by cytochrome P450 and conjugation by GST. Both acrylamide and glycidamide can combine with DNA and cause genotoxicity.⁵

From the national dietary survey in Japan in 2012, acrylamide intake was estimated by Monte Carlo simulation to be 0.166 µg/kg bodyweight per day.⁶ This level is less than half of that reported in Western populations, namely 0.45 µg/kg bodyweight per day in the Dutch⁷ and 0.41 µg/kg bodyweight per day in Norwegians.⁸ These levels are lower than in animal studies;^{9,10} however, when the benchmark dose lower confidence limit (BMDL₁₀) is 0.31 mg/kg bodyweight per day for mammary tumors in rats, the MOE is <10 000.² Therefore, the Food Safety Commission of Japan is vigilant about the possibility of a carcinogenic effect of dietary acrylamide.²

Currently, 8 studies have examined the relationship between dietary acrylamide exposure and breast cancer.¹¹⁻¹⁸ A recent meta-analysis of these studies observed that dietary acrylamide intake was not associated with the risk of breast cancer.¹⁹ However, these studies were all conducted in Western countries and no study has assessed the risk of acrylamide intake on breast cancer in Asians. Moreover, the meta-analysis included 7 studies, and some estimates were from stratified analyses, such as in premenopausal women¹⁶ or by hormone receptor status of breast cancer.¹⁷ The results might therefore not be robust, and further investigation among a variety of populations with various levels of acrylamide intake may be necessary.

The aim of the present study was to investigate the association between dietary acrylamide intake and the risk of breast cancer in the JPHC Study.

2 | MATERIALS AND METHODS

2.1 | Study participants

The JPHC Study is a population-based prospective study which aims to investigate the associations between lifestyle and lifestyle diseases in 2 cohorts. Cohort I was launched in 1990 in Iwate, Akita, Nagano, Okinawa-Chubu, and Tokyo, whereas Cohort II was started in 1993 in Ibaraki, Niigata, Kochi, Nagasaki, Okinawa-Miyako, and Osaka. The study protocol has been described previously.^{20,21} Participants were 140 420 inhabitants (68 722 men and 71 698 women) aged 40-69 years in the jurisdictional area of these 11 public health centers. Inhabitants in the Tokyo area were not included as participants in this study because their incidence data were not available. The study protocol was approved by the institutional review boards of the National Cancer Center, Tokyo, Japan, Osaka University and Azabu University. The authors confirm that some access restrictions apply to the data underlying the findings.

A dietary survey using a self-administered FFQ was conducted at baseline, and at 5- and 10-year follow up. The FFQ of the 5-year follow-up survey obtained more detailed dietary information than the FFQ of the baseline survey because it included more food items and portion size options than the baseline survey questionnaire. We

therefore used the 5-year follow-up survey as the starting point of the present study.

After excluding participants who were disqualified (non-Japanese nationality, incorrect late report of migration occurring before the starting point, or incorrect birth data) or had died, moved out of a study area, or were lost to follow up before the starting point, 62 750 women were eligible for participation. Of these, 52 483 women responded to the 5-year follow-up questionnaire (response rate 83.6%).

Participants with a past history of breast cancer as identified by the questionnaire (N = 478) and those diagnosed with breast cancer from the baseline survey to the time of the 5-year follow-up survey were excluded (N = 27). Participants with missing or extreme (upper and lower 2.5 percentiles) energy intake data were also excluded (N = 3068). Finally, 48 910 participants were included in the study (Figure 1).

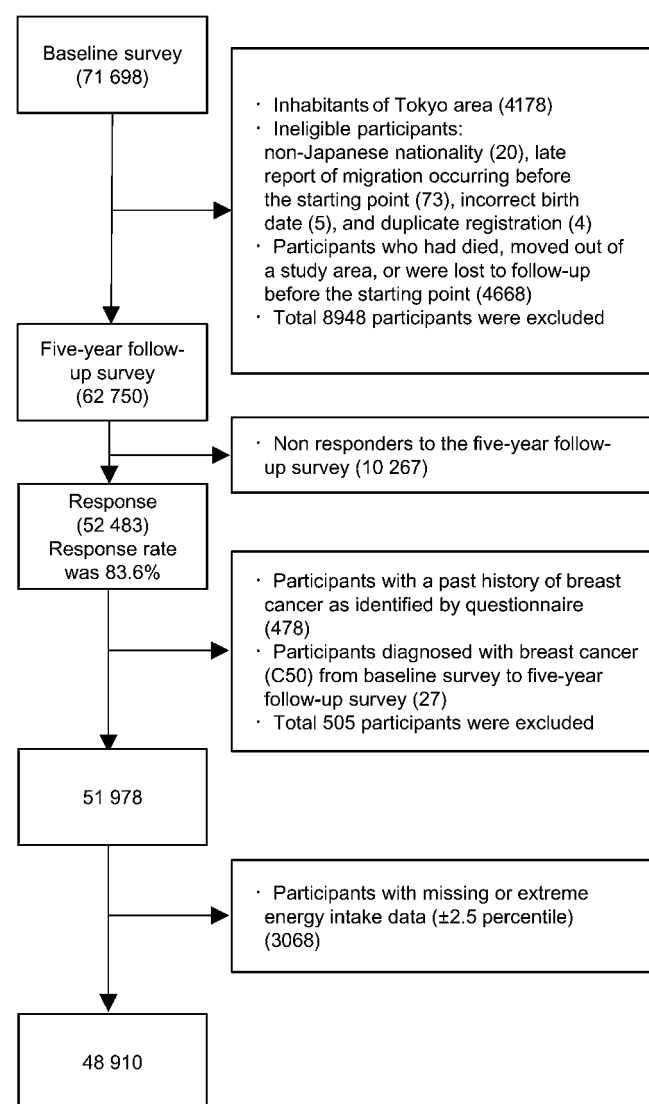


FIGURE 1 Flowchart of study participants in the Japan Public Health Center-based Prospective Study on dietary acrylamide intake and risk of breast cancer

2.2 | Assessment of energy and acrylamide intake from FFQ

The FFQ is based around a list of 138 food and beverage items, each with 9 categories of eating frequency (never, 1-3 times/mo, 1-2 times/wk, 3-4 times/wk, 5-6 times/wk, 1 time/d, 2-3 times/d, 4-6 times/d, or ≥ 7 times/d). The food items also have 3 categories of portion size (less than half the standard portion size, standard portion size, or more than 1.5-fold the standard portion size). Intake amount of each food and beverage was estimated by multiplying the eating frequency with the portion size.

Energy intake was estimated using the Fifth Revised and Enlarged Edition of the Standard Tables of Food Composition in Japan.²² A validation study of the FFQ was previously conducted by comparing intake from a 28-day DR as reference in a subcohort of the JPHC study.²³⁻²⁵ Correlation coefficients of energy intake among women were 0.41 and 0.24 in Cohort I ($n = 113$) and II ($n = 176$), respectively.²³ We previously reported the validity of acrylamide intake measurement from the FFQ using this existing data and our database of measured values of acrylamide content in common Japanese foods elsewhere.²⁶ Briefly, we developed a database of acrylamide-containing foods commonly consumed in Japan using published reports of measurements and selected the values of the following foods and beverages: miso, beer, baked fish paste, bread, rice cake, Japanese-style confectionary, cake, biscuits and cookies, chocolate, peanuts, fried tofu, green tea, oolong tea, black tea, coffee, and soup.^{6,27-35} Further, we considered the amount of acrylamide consumed from homemade cooking. Acrylamide intake from heated starchy vegetables (potato, sweet potato), vegetables (onion, bean sprouts, sweet pepper, squash, cabbage, snap beans, broccoli), toast, boiled or stir-fried rice, and fried batter was calculated by multiplying the amount of raw food, the proportion of heated food calculated from the DR and the concentration of acrylamide in each heated food.⁶ Because Cohort I and Cohort II are independently conducted studies which collected DR among different populations, we used the proportion of cooking methods among Cohort I in the calculation of acrylamide in Cohort II, or vice versa. The de-attenuated correlation coefficients of energy-adjusted acrylamide intake among women were 0.48 and 0.37 in Cohorts I and II, respectively.²⁶

2.3 | Follow up and identification of breast cancer

All participants were followed from the starting point until December 31, 2013 (until December 31, 2012 in the Osaka area only). Residence status was confirmed annually through the residential registry. During the follow-up period, 6059 (12.4%) participants died, 3330 (6.8%) moved out of the study area, and 33 (0.1%) were lost to follow up.

Incidence of breast cancer was identified through the following data sources: active patient notification from major local hospitals in

the study area and data linkage with population-based cancer registries. Death certificates were used as a supplementary information source. Cases were coded using the ICD-O-3; breast cancer is C500-509. The proportion of cases ascertained by DCO was 1.9%. This percentage was considered satisfactory for the present study. With a mean follow-up period of 15.4 years, a total of 792 newly diagnosed breast cancer cases were identified by December 31, 2013.

2.4 | Statistical analysis

Person-years of follow up were determined from the starting point until the date of diagnosis of breast cancer, date of death, date of relocation from the study area, or end of the study period (December 31, 2012 for the Osaka area and December 31, 2013 for other areas), whichever occurred first. For participants lost to follow up, data were censored on the last confirmed date of presence in the study area.

A Cox proportional hazards model was used to estimate HR and 95% CI of breast cancer by tertile of dietary acrylamide intake, using the lowest (T1) versus the middle (T2) or highest (T3) group. Trends were assessed by assignment of ordinal values for tertile of dietary acrylamide intake. For further analysis, 9 quantiles were also used in the Cox proportional hazards model. Acrylamide intake was adjusted for energy intake using the residual method. HR were adjusted for the following potential confounding factors: age, PHC area, smoking status (current, past, never, or missing), alcohol intake (<150 g/wk or ≥ 150 g/wk), BMI (<25 , ≥ 25 , or missing), family history of breast cancer (yes or no), age at menarche (≤ 13 , 14, 15, ≥ 16 , or missing), age at first delivery (<26 , ≥ 26 , or missing), number of deliveries (0, 1-2, 3, ≥ 4 , or missing), menopausal status and age at menopause (premenopause, postmenopause from age <49 , postmenopause from age 50 to 54, postmenopause from >55 , or missing) and exogenous hormone use (yes, no, or missing). These variables were obtained from the questionnaire, and are known or suspected risk factors for breast cancer in the JPHC study. Further, we also adjusted for physical activity (metabolic equivalents) and isoflavone intake; as the results did not substantially change, however, we did not use these variables for adjustment in the final model. In a sensitivity analysis, we repeated the same analysis after excluding 120 breast cancer cases diagnosed in the first 3 years of follow up.

To elucidate the interaction effect, we conducted stratified analysis by smoking status (current smoker, past smoker, or never smoker), coffee consumption (<1 cup/wk, 1 cup or more/wk), alcohol consumption (<150 g/wk or ≥ 150 g/wk), BMI (<25 or ≥ 25), and menopausal status at starting point (pre or postmenopause). A further stratified analysis was conducted for tumor subtype defined by ER/PR status, namely ER+, ER-, PR+, PR-, ER+/PR+, and ER-/PR-. All *P*-values were 2-sided and statistical significance level was set at $P < .05$ using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

3 | RESULTS

3.1 | Characteristics of participants

Table 1 shows participant characteristics according to acrylamide intake. Mean (SD) dietary acrylamide intake overall was 7.0 (3.7) µg/d, corresponding to 0.14 (0.13) µg/kg bodyweight/day. There were significant differences in the following characteristics between tertiles. The highest acrylamide intake group tended to be younger and have a lower BMI; have a higher proportion of current smoking, younger menarche, premenopausal status, and exogenous female hormone non-use; have a lower proportion of older first delivery, and non- or few deliveries; and to consume less alcohol, and more coffee, green tea, biscuits and cookies, potatoes, and vegetables. There was no significant difference between tertiles in the proportion of a family history of breast cancer.

Figure 2 shows the contribution of acrylamide-containing foods among the total study population. The food group with the greatest contribution was beverages (total 49%; 24% for coffee, 23% for green tea, and 2% for others), followed by confectioneries (total 19%; 13% for biscuits and cookies, 3% for chocolate, and 3% for others), potatoes and starches (total 13%; 12% for potatoes and 1% for others), vegetables (total 11%; 4% for sweet pepper, 3% for onion, 3% for bean sprouts, and 1% for others), and cereals (total 6%; 3% for rice and 3% for others). The main contributing foods were common in each acrylamide intake group, but the trend slightly differed (Figure 3). As acrylamide intake increased, the contribution from coffee, green tea, and biscuits and cookies increased, whereas that from potatoes, vegetables and rice decreased.

3.2 | Association between dietary acrylamide intake and breast cancer

Table 2 shows the results of dietary acrylamide intake and risk of breast cancer. There was no association between dietary acrylamide intake and breast cancer. Compared to the lowest group, HR (95% CI) was 1.00 (0.84-1.18) in the middle group and 0.95 (0.79-1.14) in the highest (*P* for trend = .58). This result was consistent with the results obtained when cases occurring within 3 years after the start of follow up were excluded.

To clarify the risk in extremely high dietary acrylamide consumers among these study participants, we conducted a further analysis between 9 quantiles of acrylamide intake (Figure 4). Mean (SD) dietary acrylamide intake was 2.5 (0.7) µg/d among the lowest 9 quantile consumers and 14.6 (3.6) among the highest 9 quantile consumers. No significant association was observed. Compared to the lowest quantile, HR (95% CI) of the highest quantile was 0.91 (0.66-1.25) and *P* for trend was .81.

Although we also conducted stratified analyses by major confounding factors, significance associations were not observed among current or past smokers (*P* for trend = .64), never smokers (*P* for trend = .43), lower coffee consumers (*P* for trend = .58), coffee consumers (*P* for trend = .71), lower alcohol consumers (*P* for

TABLE 1 Characteristics of the study participants

	Tertile of acrylamide intake			P-value ^a
	Lowest (T1)	Middle (T2)	Highest (T3)	
Number of participants	16 303	16 304	16 303	
Acrylamide intake				
Range, µg/d ^b	0.0-5.1	5.1-7.8	7.8-63.0	
Mean and SD, µg/d ^b	3.6 ± 1.0	6.3 ± 0.8	11.1 ± 3.3	
Mean and SD, µg/kg bodyweight per day ^b	0.07 ± 0.06	0.12 ± 0.11	0.22 ± 0.16	
Age at 5-y follow-up survey, year	58 ± 8	57 ± 8	55 ± 8	<.001
Body mass index, kg/m ²	24 ± 3	23 ± 3	23 ± 3	<.001
Smoking status, %				<.001
Current	4.0	4.4	7.0	
Past	0.8	1.0	1.2	
Never	88.1	88.3	85.6	
Missing	7.0	6.4	6.1	
Family history of breast cancer, %				.482
Yes	0.9	1.1	1.0	
No	99.1	99.0	99.0	
Age at menarche, years				<.001
≤13	18.5	23.7	27.2	
14	18.8	21.4	22.0	
15	19.1	18.8	17.2	
≥16	28.6	24.0	20.2	
Missing	15.0	12.1	13.4	
Age at first delivery, %				<.001
<26 y	50.6	51.1	48.9	
≥26 y	25.8	28.5	29.7	
Missing	23.6	20.4	21.4	
Number of deliveries, %				<.001
None	4.9	5.5	5.6	
1-2	32.9	36.1	36.4	
3 times	23.6	24.6	23.8	
≥4 times	19.7	18.5	18.0	
Missing	19.0	15.4	16.2	

(Continues)

trend = .52), higher alcohol consumers (*P* for trend = .60), women with a normal BMI (*P* for trend = .62), obese women (*P* for trend = .74), premenopausal women (*P* for trend = .37), or postmenopausal women (*P* for trend = .97). Further, when stratified by estrogen receptor and progesterone receptor status, there were no significant associations among ER+ (*P* for trend = .99), ER– (*P* for trend = .48), PR+ (*P* for trend = .91), PR– (*P* for trend = .33), ER+/

TABLE 1 (Continued)

	Tertile of acrylamide intake			P-value ^a
	Lowest (T1)	Middle (T2)	Highest (T3)	
Menopausal status, %				<.001
Pre-menopause	15.3	21.0	28.3	
Post-menopause from unknown age	2.0	1.6	1.5	
Post-menopause from age <49 y	37.1	34.9	33.1	
Post-menopause from age 50-54 y	35.9	36.0	31.7	
Post-menopause from age >55 y	4.7	3.8	3.3	
Missing	5.0	2.7	2.1	
Exogenous hormone use, %				<.001
Yes	2.6	2.5	2.7	
No	89.4	92.7	93.1	
Missing	8.1	4.9	4.2	
Dietary intake				
Energy, kcal/d	1838 ± 576	1866 ± 555	1848 ± 562	<.001
Alcohol intake, g/wk	16 ± 80	13 ± 58	14 ± 55	<.001
Coffee, g/d ^b	36 ± 50	91 ± 90	230 ± 240	<.001
Green tea, g/d ^b	342 ± 352	543 ± 430	798 ± 705	<.001
Biscuits and cookies, g/d ^b	1 ± 1	2 ± 3	6 ± 8	<.001
Potatoes, g/d ^b	12 ± 10	19 ± 15	23 ± 24	<.001
Vegetables, g/d ^b	202 ± 122	230 ± 124	234 ± 136	<.001

Data represent mean (standard deviation) or percentages.

^aKruskal-Wallis test for continuous variables and chi-squared test for categorical variables.

^bEnergy adjusted intake by residual method.

PR+ (*P* for trend = .92), or ER-/PR- (*P* for trend = .35) (Table 2). Additionally, there were no significant associations when stratified by green tea intake (data not shown).

4 | DISCUSSION

We found that dietary acrylamide intake was not associated with breast cancer risk in a large prospective cohort study among

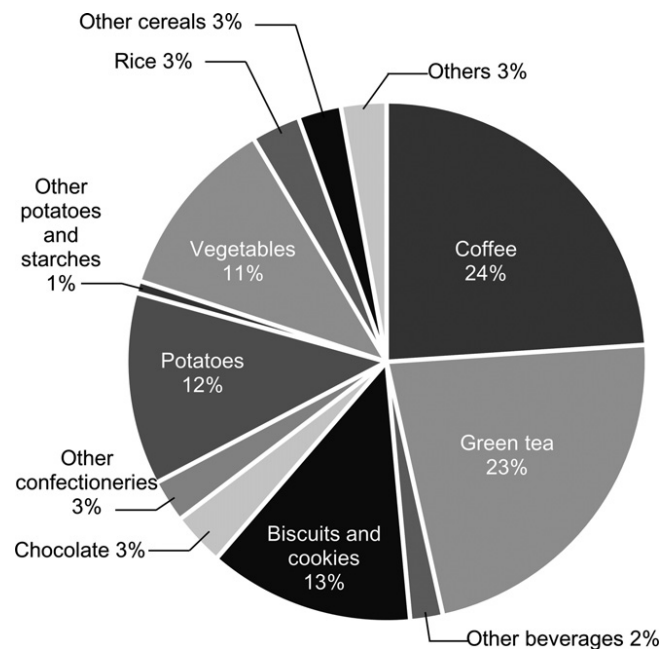


FIGURE 2 Contribution of acrylamide-containing foods among all participants

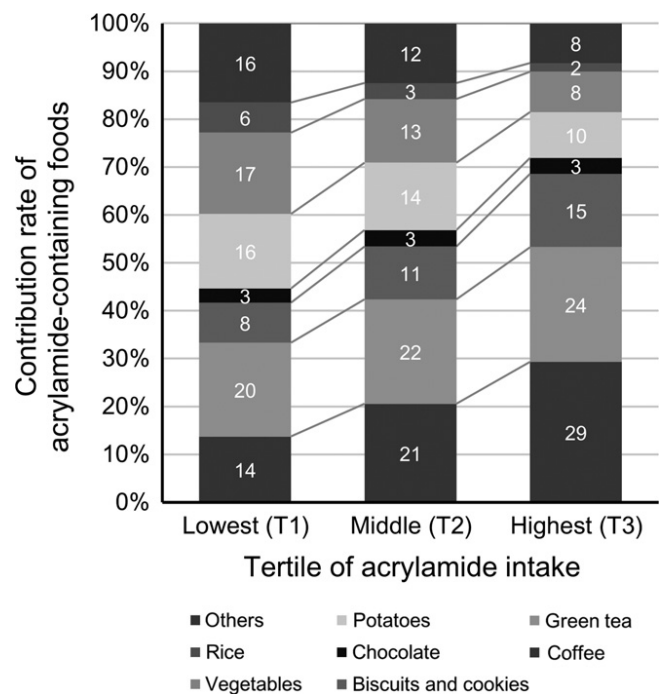


FIGURE 3 Comparison of the contribution of acrylamide-containing foods between tertiles of acrylamide intake

Japanese women. In addition, we also found no associations when stratified analyses were conducted by smoking status, coffee consumption, alcohol consumption, BMI, menopausal status, or the hormone receptor status of breast cancer tumors.

These results showing no association between dietary acrylamide intake and breast cancer are consistent with the results of a meta-analysis by Pelucchi et al of 5 prospective cohort studies, one case-

TABLE 2 Acrylamide intake and risk of breast cancer

		Tertile of acrylamide intake						P for trend
		Lowest (T1)		Middle (T2)		Highest (T3)		
		HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	
Total								
All women								
No. participants	48 910	16 303		16 304		16 303		
No. cases	792	266		268		258		
Person-years	754 623	253 736		251 712		249 176		
Age- and area-adjusted		1.00	(Reference)	1.00	(0.84-1.19)	0.95	(0.79-1.13)	.55
Multivariate-adjusted ^a		1.00	(Reference)	1.00	(0.84-1.18)	0.95	(0.79-1.14)	.58
Multivariate-adjusted (excluding cases <3 y) ^a		1.00	(Reference)	1.05	(0.87-1.26)	0.96	(0.79-1.17)	.70
By smoking status								
Current or past smoker								
No. participants	3014	796		871		1347		
No. cases	46	15		12		19		
Person-years	43 381	11 598		12 452		19 332		
Multivariate-adjusted ^a		1.00	(Reference)	0.77	(0.35-1.66)	0.83	(0.41-1.70)	.64
Never smoker								
Number of participants	42 708	14 359		14 388		13 961		
Number of cases	701	239		238		224		
Person-years	666 754	226 260		224 636		215 859		
Multivariate-adjusted ^a		1.00	(Reference)	0.97	(0.81-1.17)	0.93	(0.77-1.12)	.43
By coffee consumption								
<1 cup/wk								
No. participants	13 967	8003		3731		2233		
No. cases	206	121		62		23		
Person-years	213 780	123 302		56 865		33 613		
Multivariate-adjusted ^a		1.00	(Reference)	1.19	(0.87-1.62)	0.77	(0.49-1.20)	.58
1 cup or more/wk								
No. participants	34 943	8300		12 573		14 070		
No. cases	586	145		206		235		
Person-years	540 843	130 434		194 847		215 563		
Multivariate-adjusted ^a		1.00	(Reference)	0.93	(0.75-1.16)	0.95	(0.77-1.18)	.71
By alcohol consumption								
<150 g/wk								
No. participants	47 536	15 800		15 887		15 849		
No. cases	757	254		258		245		
Person-years	734 543	246 259		245 643		242 641		
Multivariate-adjusted ^a		1.00	(Reference)	1.00	(0.84-1.19)	0.94	(0.78-1.13)	.52
≥150 g/wk								
No. participants	1374	503		417		454		
No. cases	35	12		10		13		
Person-years	20 081	7477		6068		6535		
Multivariate-adjusted ^a		1.00	(Reference)	0.94	(0.38-2.30)	1.26	(0.53-3.01)	.60
By BMI								
<25 kg/m ²								
No. participants	34 090	11 012		11 344		11 734		
No. cases	506	163		173		170		

(Continues)

TABLE 2 (Continued)

		Tertile of acrylamide intake						P for trend
		Lowest (T1)		Middle (T2)		Highest (T3)		
	Total	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	
Person-years	524 930	171 394		174 710		178 825		
Multivariate-adjusted ^a		1.00	(Reference)	1.01	(0.82-1.26)	0.95	(0.76-1.18)	.62
≥25 kg/m ²								
No. participants	13 495	4754		4551		4190		
No. cases	266	97		85		84		
Person-years	211 475	75 034		71 292		65 149		
Multivariate-adjusted ^a		1.00	(Reference)	0.89	(0.66-1.20)	0.95	(0.70-1.29)	.74
By menopausal status								
Premenopause								
No. participants	10 523	2493		3422		4608		
No. cases	201	52		72		77		
Person-years	166 575	39 997		54 362		72 216		
Multivariate-adjusted ^a		1.00	(Reference)	1.04	(0.73-1.49)	0.86	(0.60-1.24)	.37
Postmenopause								
No. participants	36 803	13 000		12 450		11 353		
No. cases	572	203		193		176		
Person-years	564 230	201 595		190 725		171 910		
Multivariate-adjusted ^a		1.00	(Reference)	1.00	(0.82-1.22)	1.00	(0.81-1.23)	.97
By hormone receptor status								
ER+								
No. subjects	48 344	16 113		16 117		16 114		
No. cases	226	76		81		69		
Person-years	749 403	252 011		249 953		247 439		
Multivariate-adjusted ^a		1.00	(Reference)	1.11	(0.81-1.52)	1.00	(0.71-1.40)	.99
ER−								
No. subjects	48 218	16 074		16 069		16 075		
No. cases	100	37		33		30		
Person-years	748 275	251 669		249 587		247 020		
Multivariate-adjusted ^a		1.00	(Reference)	0.92	(0.57-1.49)	0.83	(0.51-1.38)	.48
PR+								
No. subjects	48 287	16 093		16 096		16 098		
No. cases	169	56		60		53		
Person-years	748 965	251 875		249 815		247 275		
Multivariate-adjusted ^a		1.00	(Reference)	1.10	(0.76-1.59)	1.02	(0.69-1.50)	.91
PR−								
No. subjects	48 268	16 094		16 086		16 088		
No. cases	150	57		50		43		
Person-years	748 702	251 820		249 715		247 167		
Multivariate-adjusted ^a		1.00	(Reference)	0.93	(0.63-1.37)	0.82	(0.54-1.23)	.33
ER+/PR+								
No. subjects	48 277	16 089		16 095		16 093		
No. cases	159	52		59		48		
Person-years	748 933	251 862		249 808		247 262		
Multivariate-adjusted ^a		1.00	(Reference)	1.18	(0.81-1.72)	1.02	(0.68-1.52)	.92

(Continues)

TABLE 2 (Continued)

		Tertile of acrylamide intake						P for trend
		Lowest (T1)		Middle (T2)		Highest (T3)		
		HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	
ER+/PR-		Total						
No. subjects	48 205	16 070		16 067		16 068		
No. cases	87	33		31		23		
Person-years	748 237	251 657		249 575		247 005		
Multivariate-adjusted ^a			1.00 (Reference)	1.01 (0.61-1.66)		0.76 (0.44-1.32)		.35

CI, confidence interval; ER, estrogen receptor; HR, hazard ratio; PR, progesterone receptor.
^aMultivariable Cox proportional hazard models were adjusted for age (year), area (10 public health center areas), body mass index (BMI) (<25, ≥25, or missing), family history of breast cancer (yes or no), age at menarche (≤13, 14, 15, ≥16, or missing), age at first delivery (<26, ≥26, or missing), number of deliveries (0, 1-2, 3, ≥4, or missing), menopausal status and age at menopause (premenopause, postmenopause from age <49, postmenopause from age 50 to 54, postmenopause from >55, or missing), use of exogenous female hormones (yes, no, or missing), smoking status (current or past, never, or missing), and alcohol intake (<150 g/wk or ≥150 g/wk).

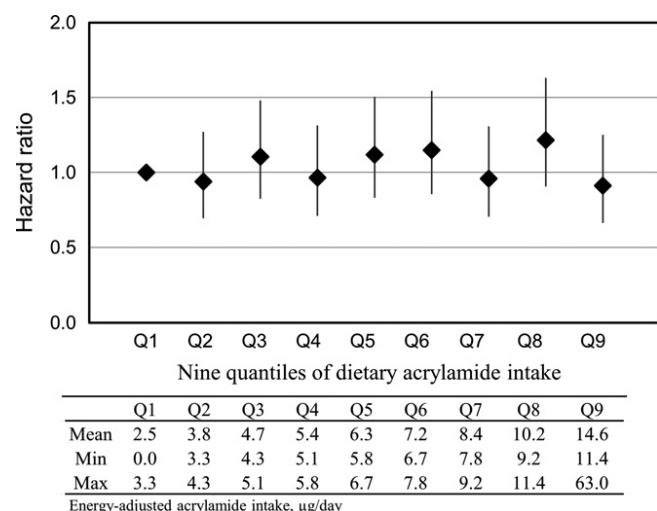


FIGURE 4 Hazard ratios (HR) of breast cancer within 9 quantiles of energy-adjusted dietary acrylamide intake. The reference group was the lowest of 9 quantiles. HR and 95% confidence intervals were adjusted for age (years), area (10 public health center areas), body mass index (<25, ≥25, or missing), family history of breast cancer (yes or no), age at menarche (≤13, 14, 15, ≥16, or missing), age at first delivery (<26, ≥26, or missing), number of deliveries (0, 1-2, 3, ≥4, or missing), menopausal status and age at menopause (premenopause, postmenopause from age <49, postmenopause from age 50 to 54, postmenopause from >55, or missing), use of exogenous female hormones (yes, no, or missing), smoking status (current or past, never, or missing), and alcohol intake (<150 g/wk or ≥150 g/wk). Numbers of cases from the lowest to the highest of 9 quantiles were 86, 83, 97, 80, 93, 95, 80, 102, and 76, respectively

control study and 2 case-cohort studies of the risk of dietary acrylamide intake for breast cancer in Western countries, which reported that there was no association between dietary acrylamide intake and breast cancer.¹⁹

The 5 prospective cohort studies and 2 case-cohort studies were conducted in Sweden, the Netherlands, the USA and the UK.^{11,13-18} Although all studies showed no association between dietary

acrylamide intake and breast cancer risk in all women, a positive association was observed in premenopausal women in the UK. The authors suggested that this positive association appears to represent a proxy for an unhealthier diet, because mean dietary acrylamide intake was less than in other countries and the main sources of dietary acrylamide intake were chips and crisps.¹⁶

In the present study, daily mean (SD) dietary acrylamide intake was 7.0 (3.7) µg in Japanese women. Western women consume 2-3 times more acrylamide than Japanese women, and mean levels of intake among Japanese women correspond to the lowest or second lowest quintile in Western women.^{11,16-18} This low and narrow intake pattern in Japanese women may affect the association toward null. Therefore, dietary acrylamide intake does not seem to increase the risk of breast cancer in Japanese women. However, 1 reason that most studies showed no association between dietary acrylamide intake and breast cancer is that country-specific analyses failed to ensure a wide distribution of intake such that the influence of dietary acrylamide could be detected.

The main sources in our Japanese population were coffee and green tea. Although green tea is specific for Japanese participants, coffee is also a common contributing food for acrylamide intake in Western countries.^{11,14,18} In a meta-analysis, coffee had a weakly preventive effect on breast cancer.³⁶ However, no preventive or causative effect was observed for our cohort between coffee or green tea intake and breast cancer risk.³⁷ Further, stratified analysis by coffee or green tea consumption indicated there was no interaction effect in our study. Although coffee/green tea is the major source of acrylamide intake in Japan, the intake of coffee/green tea did not have a causative effect on breast cancer in this study.

We also found no associations when our study participants were stratified by alcohol consumption and BMI level. When acrylamide was consumed, it is partly metabolized by CYP2E1 to glycidamide, which is a more reactive compound than acrylamide.⁵ In a cross-sectional study, the ratio of hemoglobin adduct concentrations of acrylamide to glycidamide differed according to alcohol drinking habit and BMI level, because the activity of CYP2E1 is affected by alcohol

consumption and BMI level.^{38,39} However, we could not detect any associations in a stratified analysis. As acrylamide metabolism is also affected by polymorphisms in CYP2E1, differences in the distribution of these single-nucleotide polymorphisms (SNP) may also have affected the results.^{40,41}

We also conducted stratified analysis by menopausal status and the hormone receptor status of tumors, but observed no associations between dietary acrylamide intake and breast cancer. This result is consistent with previous studies.^{14,15,17,18} However, the effect of trace acrylamide intake on hormone concentration in humans is currently under investigation and the results to date are not consistent. Hogervorst et al⁴² reported that the hemoglobin adduct concentration of acrylamide was positively associated with estrogen concentration in premenopausal American women whose BMI was <25. In contrast, Nagata et al⁴³ showed that dietary acrylamide intake assessed by FFQ was negatively related to estrogen concentration among premenopausal Japanese women. In a nested case-control study by Olsen et al,⁴⁴ the hemoglobin adduct concentration of acrylamide was positively associated with the risk of ER+ breast cancer in smokers. Therefore, further studies are needed before the effect of acrylamide intake on the hormone-related pathway can be conclusively determined.

The major strength of the present study was its prospective cohort study design. Recall bias of exposure and confounding factors was avoided because data collection was conducted before breast cancer was diagnosed. Participants were selected from the general population, the sample size was large, the response rate to the questionnaire was acceptable (83.6%) for study settings such as this, and the loss to follow up (0.1%) was negligible. The proportion of cases ascertained by DCO was 1.9%. Furthermore, the cancer registry in the study population was of sufficient quality to reduce the possibility of misclassification of outcome.

This study has several limitations. First, there is a possibility of misclassification of acrylamide intake groups. The correlation coefficients among dietary acrylamide intake from the DR and FFQ were low to moderate and kappa coefficients in quintiles were over 0.8. High kappa coefficients showed categorical agreement, but the possibility of the attenuation of relative risk by misclassification of exposure assessment still remains. Moreover, the JPHC study and the validation study for the FFQ were conducted in the 1990s, but we calculated acrylamide intake using the measured values of acrylamide in foods in the 2000s because measured values were not available in the 1990s. This time lag may have lead to underestimation and misclassification because of the efforts of food companies in reducing acrylamide content in foods. However, the concentrations of acrylamide in coffee, which was the most important food in total acrylamide intake, did not dramatically differ between the recent decades,⁶ and the effect is therefore considered to be relatively small. Second, assessment of dietary acrylamide intake by FFQ may not reflect the true acrylamide and glycidamide exposure because acrylamide metabolism may be affected by individual enzyme activity⁴⁰ and lifestyle.³⁸ Further epidemiological study using biomarkers is needed to clarify acrylamide and glycidamide exposure in terms of

internal dose. Third, the occurrence of breast cancer in Japan is less than in Western countries. Despite a reasonably large cohort population (48 910 women) and long follow-up period (average 15 years), the number of cases of breast cancer in this cohort was relatively small (n = 792), reflecting the low incidence rate in Japan (age-standardized rate per 100 000 world population in 2012, 51.5 in Japan and 92.9 in the USA for comparison).⁴⁵ The lack of subjects and cases may have rendered some null associations in the stratified analyses less robust, and interpretation may therefore need caution. Fourth, other confounding factors might have affected the results. Although we adjusted for several confounding factors in the statistical model to the maximum degree possible, the effects of unmeasured confounders cannot be totally discarded.

In conclusion, we found that there was no association between dietary acrylamide intake and breast cancer risk regardless of smoking status, coffee consumption, alcohol consumption, BMI, menopausal status, or hormone receptor status of breast cancer tumors in a large prospective cohort study among Japanese women. Our findings suggest that dietary acrylamide intake is unlikely to increase the risk of breast cancer in Japanese women.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest for this article.

AUTHORS' CONTRIBUTION

JI and TS designed the research; ST, TS, JI, NS, and MI conducted research; AK contributed to the calculation of dietary acrylamide intake; AK, LZ, and RL carried out statistical analysis; AK interpreted the results and wrote the paper; and JI had primary responsibility for final content. All authors reviewed the manuscript and contributed to the discussion.

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REFERENCES

1. IARC. IARC working group on the evaluation of carcinogenic risks to humans: some industrial chemicals. Lyon, 15-22 February 1994. *IARC Monogr Eval Carcinog Risks Hum.* 1994;60:1-560.

2. Food Safety Commission of Japan. *Evaluation Document of Dietary Acrylamide Produced by Heating*. Tokyo: Food Safety Commission of Japan; 2016. https://www.fsc.go.jp/osirase/acrylamide1.data/acrylamide_hyokasyo1.pdf Accessed January 17, 2017.
3. Tareke E, Rydberg P, Karlsson P, et al. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem*. 2002;50:4998-5006.
4. Viswanath P. Evaluation of certain contaminants in food (Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives). *Indian J Med Res*. 2012;135:795.
5. Shipp A, Lawrence G, Gentry R, et al. Acrylamide: review of toxicity data and dose-response analyses for cancer and noncancer effects. *Crit Rev Toxicol*. 2006;36:481-608.
6. Food Safety Commission of Japan. Study on estimate of acrylamide intake from food; interim report. 2016. Food Safety Commission of Japan. <https://www.fsc.go.jp/fscis/technicalResearch/show/cho99920151507> Accessed 07/01 2016.
7. Konings EJ, Hogervorst JG, van Rooij L, et al. Validation of a database on acrylamide for use in epidemiological studies. *Eur J Clin Nutr*. 2010;64:534-540.
8. Brantsaeter AL, Haugen M, Mul A, et al. Exploration of different methods to assess dietary acrylamide exposure in pregnant women participating in the Norwegian Mother and Child Cohort Study (MoBa). *Food Chem Toxicol*. 2008;46:2808-2814.
9. Beland FA, Mellick PW, Olson GR, et al. Carcinogenicity of acrylamide in B6C3F1 mice and F344/N rats from a 2-year drinking water exposure. *Food Chemical Toxicol*. 2013;51:149-159.
10. Beland FA, Olson GR, Mendoza MC, et al. Carcinogenicity of glycidamide in B6C3F1 mice and F344/N rats from a two-year drinking water exposure. *Food Chem Toxicol*. 2015;86:104-115.
11. Mucci LA, Sandin S, Bälter K, et al. Acrylamide intake and breast cancer risk in Swedish women. *JAMA*. 2005;293:1322-1327.
12. Pelucchi C, Galeone C, Levi F, et al. Dietary acrylamide and human cancer. *Int J Cancer*. 2006;118:467-471.
13. Hogervorst JG, Schouten LJ, Konings EJ, et al. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2007;16:2304-2313.
14. Larsson SC, Åkesson A, Wolk A. Long-term dietary acrylamide intake and breast cancer risk in a prospective cohort of Swedish women. *Am J Epidemiol*. 2009;169:376-381.
15. Wilson KM, Mucci LA, Cho E, et al. Dietary acrylamide intake and risk of premenopausal breast cancer. *Am J Epidemiol*. 2009;169:954-961.
16. Burley VJ, Greenwood DC, Hepworth SJ, et al. Dietary acrylamide intake and risk of breast cancer in the UK women's cohort. *Br J Cancer*. 2010;103:1749-1754.
17. Pedersen GS, Hogervorst JG, Schouten LJ, et al. Dietary acrylamide intake and estrogen and progesterone receptor-defined postmenopausal breast cancer risk. *Breast Cancer Res Treat*. 2010;122:199-210.
18. Wilson KM, Mucci LA, Rosner BA, et al. A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancers. *Cancer Epidemiol Biomarkers Prev*. 2010;19:2503-2515.
19. Pelucchi C, Bosetti C, Galeone C, et al. Dietary acrylamide and cancer risk: an updated meta-analysis. *Int J Cancer*. 2015;136:2912-2922.
20. Watanabe S, Tsugane S, Sobue T, et al. Study design and organization of the JPHC study. Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases. *J Epidemiol*. 2001;11(suppl 6):S3-S7.
21. Tsugane S, Sawada N. The JPHC study: design and some findings on the typical Japanese diet. *Jpn J Clin Oncol*. 2014;44:777-782.
22. Resource Council, Science and Technology Agency, the Government of Japan. *Standard Tables of Food Composition in Japan*, the fifth revised edition. Tokyo, Japan: Printing Bureau, Ministry of Finance; 2002.
23. Ishihara J, Inoue M, Kobayashi M, et al. Impact of the revision of a nutrient database on the validity of a self-administered food frequency questionnaire (FFQ). *J Epidemiol*. 2006;16:107-116.
24. Tsugane S, Sasaki S, Kobayashi M, et al. Validity and reproducibility of the self-administered food frequency questionnaire in the JPHC Study Cohort I: study design, conduct and participant profiles. *J Epidemiol*. 2003;13(suppl 1):S2-S12.
25. Ishihara J, Sobue T, Yamamoto S, et al. Validity and reproducibility of a self-administered food frequency questionnaire in the JPHC Study Cohort II: study design, participant profile and results in comparison with Cohort I. *J Epidemiol*. 2003;13(suppl 1):S134-S147.
26. Kotemori A, Ishihara J, Nakadate M, et al. Validity of a self-administered food frequency questionnaire for the estimation of acrylamide intake in the Japanese population: the JPHC FFQ Validation Study. *J Epidemiol*. 2017;28.
27. National Institute for Environmental Studies. Japan Study on statistical estimate of acrylamide intake from foods National Institute for Environmental Studies, Japan. <http://www.fsc.go.jp/fscis/technicalResearch/show/cho99920141408>. Accessed January 22, 2018.
28. Ministry of Agriculture, Forestry and Fisheries Risk profile sheet relating to the food safety; for acrylamide Ministry of Agriculture, Forestry and Fisheries. http://www.maff.go.jp/j/syouan/seisaku/risk_analysis/priority/pdf/150807_rp_aa.pdf Accessed 07/01 2016.
29. National Institute of Health Sciences. Acrylamide analysis in food. <http://www.mhlw.go.jp/topics/2002/11/tp1101-1a.html> Accessed 07/01 2016.
30. Mizukami Y, Kohata K, Yamaguchi Y, et al. Analysis of acrylamide in green tea by gas chromatography-mass spectrometry. *J Agric Food Chem*. 2006;54:7370-7377.
31. Takatsuki S, Nemoto S, Sasaki K, et al. Production of acrylamide in agricultural products by cooking. *J Food Hyg Soc Japan*. 2004;45:44-48.
32. Yoshida M, Ono H, Ohnishi-Kameyama M, et al. Determination of acrylamide in processed foodstuffs in Japan. *Nippon Shokuhin Kagaku Kogaku Kaishi*. 2002;49:822-825.
33. Yoshida M, Miyoshi K, Horibata K, et al. Estimation of acrylamide intake from cooked rice in Japan. *Nippon Shokuhin Kagaku Kogaku Kaishi*. 2011;58:525-530.
34. Food Safety Commission of Japan. Information clearing sheet for Acrylamide Food Safety Commission of Japan. <https://www.fsc.go.jp/fscis/attachedFile/download?retrievalId=kai20111222sfc&fileId=520> Accessed 07/01 2016.
35. FAO/WHO Health implications of Acrylamide in Food FAO/WHO. <http://www.who.int/foodsafety/publications/acrylamide-food/en/> Accessed 07/01 2016.
36. Jiang W, Wu Y, Jiang X. Coffee and caffeine intake and breast cancer risk: an updated dose-response meta-analysis of 37 published studies. *Gynecol Oncol*. 2013;129:620-629.
37. Iwasaki M, Inoue M, Sasazuki S, et al. Green tea drinking and subsequent risk of breast cancer in a population-based cohort of Japanese women. *Breast Cancer Res*. 2010;12:R88.
38. Obon-Santacana M, Lujan-Barroso L, Freisling H, et al. Dietary and lifestyle determinants of acrylamide and glycidamide hemoglobin adducts in non-smoking postmenopausal women from the EPIC cohort. *Eur J Nutr*. 2017;56:1157-1168.
39. Emery MG, Fisher JM, Chien JY, et al. CYP2E1 activity before and after weight loss in morbidly obese subjects with nonalcoholic fatty liver disease. *Hepatology*. 2003;38:428-435.
40. Duale N, Bjellaas T, Alexander J, et al. Biomarkers of human exposure to acrylamide and relation to polymorphisms in metabolizing genes. *Toxicol Sci*. 2009;108:90-99.
41. Huang YF, Chiang SY, Liou SH, et al. The modifying effect of CYP2E1, GST, and mEH genotypes on the formation of hemoglobin


- adducts of acrylamide and glycidamide in workers exposed to acrylamide. *Toxicol Lett*. 2012;215:92-99.
42. Hogervorst JG, Fortner RT, Mucci LA, et al. Associations between dietary acrylamide intake and plasma sex hormone levels. *Cancer Epidemiol Biomarkers Prev*. 2013;22:2024-2036.
43. Nagata C, Konishi K, Tamura T, et al. Associations of acrylamide intake with circulating levels of sex hormones and prolactin in premenopausal Japanese women. *Cancer Epidemiol Biomarkers Prev*. 2015;24:249-254.
44. Olesen PT, Olsen A, Frandsen H, et al. Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study. *Int J Cancer*. 2008;122:2094-2100.
45. Ervik M, Lam F, Ferlay J, Mery L, Soerjomataram I, Bray F. *Cancer Today*. Lyon, France: International Agency for Research on Cancer. Cancer Today; 2016.

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ORIGINAL ARTICLE

WILEY **Cancer Science**

Dietary acrylamide intake and the risk of endometrial or ovarian cancers in Japanese women

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A meta-analysis published in 2015 noted a marginally increased risk of endometrial and ovarian cancers in non-smoking women with dietary acrylamide intake, but only a few studies were included, and they were limited to Western countries. The aim of this study was to investigate the association between dietary acrylamide intake and endometrial or ovarian cancer risk in the Japan Public Health Center-based Prospective Study (JPHC Study). In this prospective cohort study, 47 185 participants aged 45–74 years at the follow-up starting point in the JPHC Study were enrolled. Dietary acrylamide intake was assessed using a validated food frequency questionnaire. Cox proportional hazards regression models were used to estimate hazard ratios (HR) and 95% confidence intervals (95%CI). In participants with endometrial and ovarian cancer, the average follow-up periods were 15.5 and 15.6 years, respectively, and 161 and 122 cases of endometrial and ovarian cancer were diagnosed, respectively. Energy-adjusted dietary acrylamide intake was negatively associated with endometrial cancer, but the association disappeared after adjusting for coffee consumption with an adjusted HR for the highest vs lowest tertile of 0.85 (95%CI: 0.54–1.33). No association was observed, however, for ovarian cancer (adjusted HR, 0.77; 95%CI: 0.49–1.23). Furthermore, after stratifying by smoking status, coffee consumption, alcohol consumption, body mass index, and menopause status, no association was observed. Dietary acrylamide intake was not associated with the risk of endometrial or ovarian cancer in Japanese women with a relatively lower dietary intake of acrylamide compared with Western populations.

KEYWORDS

Asia, dietary acrylamide, endometrial cancer, epidemiology, ovarian cancer

Abbreviations: CI, confidence interval; DCO, death certificate only; EPIC, European Prospective Investigation into Cancer and Nutrition; FFQ, food frequency questionnaire; HR, hazard ratio; ICD-O-3, International Classification of Diseases for Oncology, Third Edition; JPHC Study, Japan Public Health Center-based Prospective Study; NHS, Nurses' Health Study; NLCS, Netherlands Cohort Study on Diet and Cancer; SMC, Swedish Mammography Cohort.

1 | INTRODUCTION

The International Agency for Research on Cancer classified acrylamide as a probable human carcinogen (group 2A) in 1994 given the evidence for the carcinogenicity of acrylamide in animal studies.¹ In the general population, smoking was thought to be the main

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source of acrylamide exposure,² but in 2002, Swedish researchers also found acrylamide in starchy foods cooked at high temperatures (such as fried potato).³ Therefore, in Western countries, epidemiological studies have been conducted to clarify the risk of dietary acrylamide intake with regard to the incidence of cancers.

A meta-analysis published in 2015 showed no increased risk for most cancers but, due to the marginally increased risk of endometrial and ovarian cancers identified in non-smoking women, there is a need for further studies.⁴ One possible mechanism by which dietary acrylamide exerts its carcinogenic effect is thought to be via the genotoxic pathway of glycidamide, which is an acrylamide metabolite.⁵⁻⁷ Furthermore, a hormone-related pathway has also been debated.⁷⁻⁹ Thus, acrylamide is more likely to cause a non-negligible increase in the risk of endometrial and ovarian cancers through synergistic genotoxicity effects and hormone changes than with other cancers. The small number of studies included in the meta-analysis (four studies for each cancer), however, is also considered one of the reasons for this finding. Therefore, accumulation of evidence from further studies is needed.

In the Japan Public Health Center-based Prospective Study (JPHC Study), the main sources of dietary acrylamide intake based on the dietary records were coffee and green tea, followed by confectionery, vegetables, and potatoes.¹⁰ In contrast, the main sources in Western countries were potato-based foods, wheat-based products, and coffee.¹¹⁻¹³ These differences might influence the effect of dietary acrylamide on the risk of cancer in Japan. This is because coffee is also known to be a preventive factor for endometrial cancer.¹⁴ Thus, in the case of endometrial cancer, it is expected that the carcinogenic effect of acrylamide may be attenuated by the protective effect of coffee. Therefore, in order to evaluate the safety of dietary acrylamide, it is important to examine its influence on cancers in various countries with different dietary sources. Only one study in Asia, however, has assessed the influence of dietary acrylamide intake on the incidence of cancers.¹⁵

Therefore, the aim of this study was to investigate the association between dietary acrylamide intake and the incidence of endometrial or ovarian cancers in Japanese women.

2 | MATERIALS AND METHODS

2.1 | Study participants

The JPHC Study was launched in the 1990s to investigate the associations between lifestyle-related diseases in two cohorts as a population-based prospective cohort study. Cohort I areas included Iwate, Akita, Nagano, Okinawa (Chubu), and Tokyo, while cohort II areas included Ibaraki, Niigata, Kochi, Nagasaki, Okinawa (Miyako), and Osaka. The study protocol has been described elsewhere.^{16,17} Participants in the JPHC Study aged 40-69 years in these 11 areas consisted of 140 420 inhabitants (68 722 men and 71 698 women). Dietary surveys were conducted using a self-administered food frequency questionnaire (FFQ) at baseline and at the 5-year follow-up survey. The number of food items and the number of food items

with the option of portion size in the FFQ at baseline were limited. In contrast, the FFQ at 5-year follow-up contained more detailed dietary information. Thus, we treated the 5-year follow-up survey as the starting point of the follow up, and calculated dietary acrylamide intake as the exposure variable using the 5-year follow-up FFQ. The study protocol was approved by the institutional review boards of the National Cancer Center, Tokyo, Japan; Osaka University; and Azabu University. All study participants provided informed consent prior to inclusion in the study.

Participants in the Tokyo area were not included in the present study because the incidence data for cancer cases were not available. After excluding the participants who were disqualified, had died, moved out of the study area, or were lost to follow up before the starting point, 62 750 women were eligible for inclusion in this study. Of these, respondents to the 5-year follow-up survey consisted of 52 483 women (response rate, 83.6%). Furthermore, the participants who had a past history of endometrial (n = 654) or

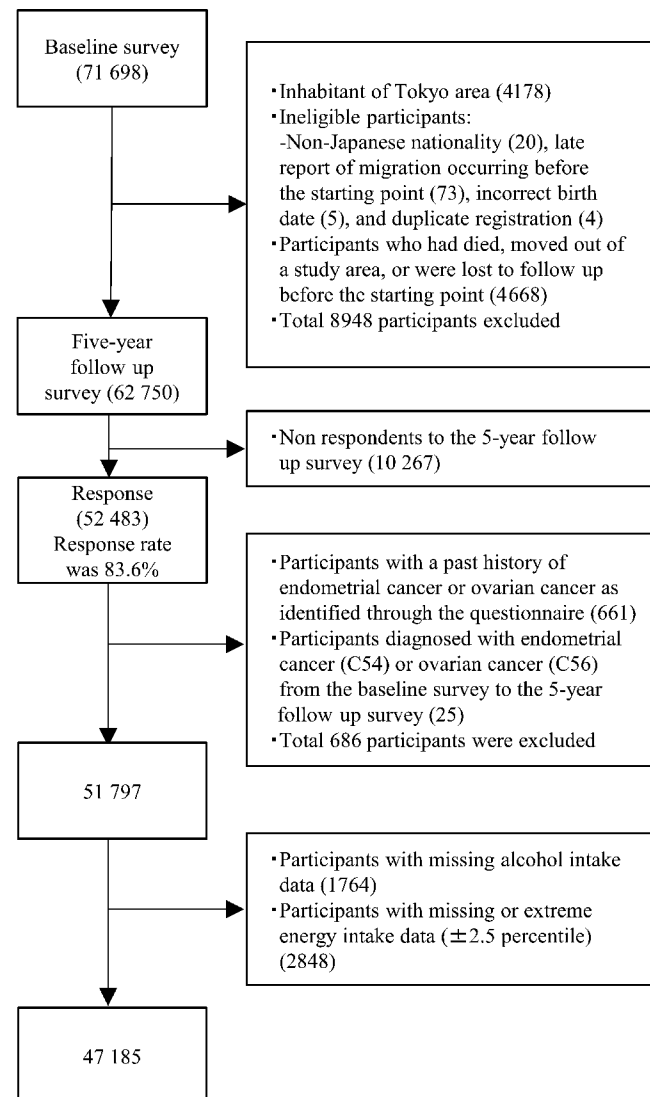


FIGURE 1 Flowchart of participant selection; Japan Public Health Center-based Prospective Study

ovarian ($n = 7$) cancers identified through the questionnaire and diagnosed during the baseline survey up to the 5-year follow-up survey were excluded (endometrial cancer, $n = 14$; ovarian cancer, $n = 11$). Participants with missing data or extreme energy intake data (upper and lower 2.5 percentile) were also excluded ($n = 4612$). Therefore, 47 185 women were included in the present analysis of endometrial and ovarian cancers (Figure 1).

2.2 | Assessment of energy and acrylamide intake from FFQ

Overall, 138 food and beverage items were included in the 5-year follow-up FFQ. The options for each food item were grouped into 9 categories according to the frequency of eating (never; 1-3 times/month; 1-2 times/week; 3-4 times/week; 5-6 times/week; once/day; 2-3 times/day; 4-6 times/day; or ≥ 7 times/day) and 3 categories according to portion size (less than half the standard portion size; standard portion size; or >1.5 -fold the standard portion size). The options for each drink were grouped into 9 categories according to the frequency of drinking (<1 cup/week; 1-2 cups/week; 3-4 cups/week; 5-6 cups/week; 1 cup/day; 1-3 cups/day; 4-6 cups/day; 7-9 cups/day; or ≥ 10 cups/day).

Energy content in each food item was referenced from the fifth revised and enlarged edition of the Standard Tables of Food Composition in Japan.¹⁸ The estimated energy intake was calculated as the sum of the product of the eating frequency, portion size, and energy content of each food. From validation studies, on comparison of 28-day dietary record (DR) in subsamples of two cohorts, the correlation coefficients of energy intake in women were 0.41 and 0.24 in cohort I ($n = 113$) and cohort II ($n = 176$), respectively.¹⁹⁻²¹

Acrylamide intake was also estimated from the amount of food and beverage intake and the acrylamide content database. The database was developed from published reports of measurements of common Japanese foods.²²⁻³¹ In addition to heated processed foods such as bread, biscuit and cookies, or coffee, we considered the influence of home cooking such as stir-fried vegetables, toast, or fried batter, to estimate the dietary acrylamide more accurately.²³ The de-attenuated correlation coefficients of energy-adjusted dietary acrylamide intake in women were 0.48 and 0.37 in cohort I and cohort II, respectively.¹⁰

2.3 | Follow up and identification of endometrial and ovarian cancers

The follow-up period for all participants was from the starting point of the 5-year follow-up survey until 31 December 2013 (until 31 December 2012, only in the Osaka area). Residential status was confirmed annually through the residential registry. During the follow-up period, 5741 participants (12.2%) died, 3191 (6.8%) moved from the study area, and 31 (0.1%) were lost to follow up.

The cancer incidence was identified through the following data sources: active patient notification from major local hospitals in the

study area and data linkage with population-based cancer registries. Death certificates were used as a supplementary information source. The International Classification of Diseases for Oncology, Third Edition (ICD-O-3) was used for coding endometrial (C54) and ovarian (C56) cancer cases. The proportion of cases determined using death certificate only (DCO) was 1.9% and 7.4% for endometrial and ovarian cancers, respectively. Given that these percentages were $<10\%$, they were considered satisfactory for the present study.³² A total of 161 endometrial and 122 ovarian cancers were newly diagnosed by 31 December 2013.

2.4 | Statistical analysis

Person-years of follow up were determined from the starting point until the date of diagnosis of endometrial or ovarian cancer, the date of a participant's death, the date of relocation from the study area, or the end of the study period (31 December 2012 for Osaka area and 31 December 2013 for all other areas), whichever occurred first. For participants lost to follow up, the last confirmed date of their presence in the study area was used as the date of censor. The mean follow-up period was 15.5 and 15.6 years for endometrial and for ovarian cancer analysis, respectively.

Cox proportional hazard modeling was used to estimate the hazard ratio (HR) and 95% confidence intervals (95%CI) to determine the association between tertiles of energy-adjusted dietary acrylamide intake and endometrial or ovarian cancers. For energy adjustment, the residual method was used. The trend of HR was also assessed using the ordinal values of the tertiles of energy-adjusted dietary acrylamide intake. HR were adjusted for the following potential confounders in model 1 for endometrial cancer analysis: age (years), area (10 public health center areas), body mass index (BMI; <25 , ≥ 25 kg/m², or missing), age at menarche (≤ 13 , 14, 15, ≥ 16 years, or missing), age at first delivery (<26 , ≥ 26 years, or missing), number of deliveries (0, 1-2, 3, ≥ 4 , or missing), menopause status and age at menopause (premenopause, postmenopause [age <49 , 50-54, ≥ 55 years], or missing), use of exogenous female hormones (yes, no, or missing), smoking status (current, ever, never, or missing), and alcohol intake (<150 or ≥ 150 g/week). Furthermore, in the multivariate-adjusted model 2, energy-adjusted coffee intake (continuous) was adjusted for in addition to the variables in model 1.

For ovarian cancer analysis, similar confounding factors as those for endometrial cancer were also adjusted for. These variables were identified from the FFQ and are known or suspected risk factors of endometrial or ovarian cancers.^{33,34} For sensitivity analysis in model 2, we repeated the same analysis for each cancer but excluded cancer cases diagnosed at ≤ 3 years of follow-up. Further, we conducted a stratified analysis using smoking status, frequency of coffee consumption (<1 cup/week, ≥ 1 cup/week), alcohol consumption, BMI, and menopause status at starting point (pre- or postmenopause). All P -values were two-sided, and statistical significance level was set at $P < .05$ using SAS 9.3 (SAS Institute, Cary, NC, USA).

TABLE 1 Subject characteristics for endometrial and ovarian cancer analysis

	Tertiles of acrylamide intake			P-value ^a
	Lowest T1 Mean \pm SD or %	Middle T2 Mean \pm SD or %	Highest T3 Mean \pm SD or %	
No. participants	15 728	15 729	15 728	
Acrylamide intake				
Median (μ g/day) ^b	3.9	6.3	10.2	
Range (μ g/day) ^b	0.0-5.1	5.1-7.9	7.9-59.0	
Mean (μ g/day) ^b	3.7 \pm 1.0	6.4 \pm 0.8	11.1 \pm 3.3	
Mean (μ g/kg bodyweight/day) ^b	0.07 \pm 0.05	0.12 \pm 0.11	0.22 \pm 0.15	
Age at 5-year follow-up survey (years)	58 \pm 8	57 \pm 8	55 \pm 8	<.001
Body mass index (kg/m ²)	24 \pm 3	23 \pm 3	23 \pm 3	<.001
Smoking status				
Current	4.1	4.5	7.2	<.001
Past	0.9	1.0	1.2	
Never	89.6	89.3	86.7	
Missing	5.5	5.3	5.0	
Menarche age				
\leq 13 years	18.8	24.1	27.7	<.001
14 years	18.9	21.7	22.2	
15 years	19.1	18.7	17.1	
\geq 16 years	28.3	23.4	19.6	
Missing	14.9	12.2	13.4	
Age at first delivery				
<26 years	50.7	50.9	48.5	<.001
\geq 26 years	25.9	28.8	30.0	
Missing	23.4	20.3	21.5	
No. deliveries				
None	4.8	5.5	5.7	<.001
1-2	33.4	36.5	37.0	
3	23.7	24.8	23.7	
\geq 4	19.4	17.9	17.5	
Missing	18.8	15.4	16.1	
Menopause status				
Premenopause	15.7	21.7	29.0	<.001
Postmenopause from unknown age	2.0	1.5	1.5	
Postmenopause from age <49 years	36.9	34.6	32.6	
Postmenopause from age 50-54 years	36.2	36.2	31.8	
Postmenopause from age \geq 55 years	4.6	3.7	3.2	
Missing	4.6	2.3	2.0	
Exogenous hormone use				
Yes	2.7	2.5	2.8	<.001
No	89.9	93.1	93.5	
Missing	7.4	4.4	3.7	
Dietary intake				
Energy (kcal/day)	1845 \pm 570	1874 \pm 553	1858 \pm 560	<.001
Alcohol intake (g/week)	16 \pm 80	13 \pm 58	14 \pm 55	<.001
Coffee (g/day) ^b	37 \pm 51	92 \pm 90	232 \pm 238	<.001

(Continues)

TABLE 1 (Continued)

	Tertiles of acrylamide intake			P-value ^a
	Lowest T1 Mean \pm SD or %	Middle T2 Mean \pm SD or %	Highest T3 Mean \pm SD or %	
Green tea (g/day) ^b	343 \pm 352	543 \pm 429	791 \pm 698	<.001
Biscuit and cookies (g/day) ^b	1 \pm 1	2 \pm 3	6 \pm 8	<.001
Potato (g/day) ^b	12 \pm 10	19 \pm 15	23 \pm 24	<.001
Vegetables (g/day) ^b	204 \pm 123	231 \pm 124	235 \pm 135	<.001

^aKruskal-Wallis test for continuous variables and chi-squared test for categorical variables.

^bEnergy-adjusted intake.

3 | RESULTS

3.1 | Participant characteristics

Participant characteristics according to acrylamide intake are listed in Table 1. The mean \pm SD dietary acrylamide intake was 3.7 \pm 1.0, 6.4 \pm 0.8, and 11.1 \pm 3.3 μ g/day in the lowest, middle, and highest tertiles of dietary acrylamide intake, respectively. Overall, the median dietary acrylamide intake was 6.3 μ g/day (IQR, 4.5-8.8 μ g/day), and the mean \pm SD dietary acrylamide intake was 7.1 \pm 3.7 μ g/day and 0.14 \pm 0.13 μ g/kg bodyweight/day in all participants. Foods that highly contributed to the total acrylamide intake were coffee (24%), green tea (22%), biscuit and cookies (13%), potatoes (12%), and vegetables (11%). When the percentages of contributing food were compared between tertiles, coffee (from 14% in the lowest to 29% in the highest), green tea (from 20% in the lowest to 24% in the highest), and biscuit and cookies (from 8% in the lowest to 15% in the highest) increased linearly. The percentages of potatoes (from 16% in the lowest to 10% in the highest) and vegetables (from 17% in the lowest to 8% in the highest), however, decreased linearly.

The highest acrylamide consumption group were younger; had lower BMI; had a larger proportion of current smokers, higher proportion of younger menarche, lower proportion of older first delivery, lower proportion of none or few deliveries, higher proportion of premenopause status, higher proportion of exogenous female hormone non-users; and consumed a higher energy diet, less alcohol, more coffee, more green tea, more biscuit and cookies, more potatoes, and more vegetables than the lowest acrylamide consumption group.

3.2 | Dietary acrylamide intake and endometrial or ovarian cancers

The association between dietary acrylamide intake and endometrial cancer risk is shown in Table 2. In model 1, dietary acrylamide intake significantly decreased the risk of endometrial cancer. When cancer cases occurring \leq 3 years of the starting point were excluded, the risk did not change. In addition to the covariates in model 1, however, we added coffee consumption in model 2; the association attenuated and no significant association was observed. This did not change after excluding cases that occurred \leq 3 years of the starting

point. Furthermore, although similar associations were observed when stratified according to the confounding factors, there was no significant association in model 2.

The associations between dietary acrylamide intake and ovarian cancer risk are given in Table 3. In contrast to the endometrial cancer analysis, no significant association was observed with ovarian cancer. Furthermore, no significant associations were seen on stratification in any of the strata.

Given the wide range of dietary acrylamide intake in the highest group (7.9-59 μ g/day), we divided all participants into 9 quantiles and conducted further analysis to clarify the risk of extremely high consumption (Figure 2). Mean dietary acrylamide intake increased by approximately 1 μ g/day between quantiles. No significant association was observed when the highest quantile was compared with the lowest quantile: HR was 0.55 (95%CI: 0.23-1.33) and *P* for trend was 0.18 for endometrial cancer risk; with 0.66 (95%CI: 0.25-1.73) and *P* for trend = 0.32 for ovarian cancer risk.

4 | DISCUSSION

This study identified no associations between dietary acrylamide intake and endometrial or ovarian cancer risks in Japanese women. Specifically, energy-adjusted dietary acrylamide intake was inversely associated with endometrial cancer in model 1, but the significant association disappeared after adjustment for coffee consumption. Furthermore, no associations were observed in either cancers after smoking status, coffee consumption, alcohol consumption, BMI, and menopause status stratifications.

In addition to the null association in all women, we did not detect any significant associations between dietary acrylamide intake and endometrial or ovarian cancers in non-smoking Japanese women. Furthermore, the point estimates showed no increase. In the previous meta-analysis, a non-negligible association was observed in non-smoking women with endometrial cancer (HR, 1.23; 95%CI: 1.00-1.51) and with ovarian cancer (HR, 1.39; 95%CI: 0.97-2.00).⁴ Moreover, the Netherlands Cohort Study on Diet and Cancer (NLCS), the Nurses' Health Study (NHS), and the European Prospective Investigation into Cancer and Nutrition (EPIC) noted increased HR for endometrial and ovarian cancers.³⁵⁻³⁷ In contrast, the Swedish Mammography Cohort (SMC) and the Italian Case-Control

TABLE 2 Acrylamide intake and the risk of endometrial cancer

		Tertiles of acrylamide intake			P for trend
		Total	Lowest (T1) HR (95%CI)	Middle (T2) HR (95%CI)	
All women					
No. participants	47 185	15 728	15 729	15 728	
No. cases	161	67	51	43	
Person-years	733 067	246 682	244 634	241 751	
Age- and area-adjusted		1.00 (Reference)	0.77 (0.53-1.12)	0.64 (0.43-0.96)	.03
Multivariate-adjusted ^a		1.00 (Reference)	0.76 (0.53-1.10)	0.65 (0.44-0.97)	.03
Multivariate-adjusted (excluding cases ≤ 3 years) ^a		1.00 (Reference)	0.79 (0.53-1.18)	0.68 (0.44-1.05)	.08
Multivariate-adjusted ^b		1.00 (Reference)	0.83 (0.57-1.22)	0.85 (0.54-1.33)	.43
Multivariate-adjusted (excluding cases ≤ 3 years) ^b		1.00 (Reference)	0.85 (0.56-1.28)	0.85 (0.52-1.38)	.46
By smoking status					
Current or past smoker					
No. cases	5	1	0	4	
Multivariate-adjusted ^a		1.00 (Reference)	–	1.68 (0.12-22.83)	.49
Never smoker					
No. cases	149	64	48	37	
Multivariate-adjusted ^a		1.00 (Reference)	0.77 (0.52-1.12)	0.62 (0.40-0.94)	.02
Multivariate-adjusted ^b		1.00 (Reference)	0.85 (0.57-1.25)	0.82 (0.51-1.31)	.37
By coffee consumption					
<1 cup/week					
No. cases	47	32	7	8	
Multivariate-adjusted ^a		1.00 (Reference)	0.51 (0.22-1.18)	1.14 (0.52-2.53)	.77
≥1 cup/week					
No. cases	114	35	44	35	
Multivariate-adjusted ^a		1.00 (Reference)	0.83 (0.53-1.31)	0.59 (0.36-0.96)	.03
Multivariate-adjusted ^b		1.00 (Reference)	0.91 (0.58-1.44)	0.79 (0.46-1.36)	.40
By alcohol consumption					
<150 g/week					
No. cases	157	64	51	42	
Multivariate-adjusted ^a		1.00 (Reference)	0.80 (0.55-1.16)	0.67 (0.44-1.00)	.05
Multivariate-adjusted ^b		1.00 (Reference)	0.88 (0.60-1.29)	0.89 (0.57-1.40)	.58
≥150 g/week					
No. cases	4	3	0	1	
Multivariate-adjusted ^a		1.00 (Reference)	–	–	–
By body mass index					
<25 kg/m ²					
No. cases	103	40	35	28	
Multivariate-adjusted ^a		1.00 (Reference)	0.87 (0.55-1.39)	0.72 (0.43-1.19)	.20
≥25 kg/m ²					
No. cases	55	25	16	14	
Multivariate-adjusted ^a		1.00 (Reference)	0.67 (0.35-1.26)	0.57 (0.29-1.13)	.10
By menopause status					
Premenopause					
No. cases	49	17	14	18	
Multivariate-adjusted ^a		1.00 (Reference)	0.62 (0.30-1.27)	0.67 (0.33-1.35)	.29

(Continues)

TABLE 2 (Continued)

	Total	Tertiles of acrylamide intake			P for trend
		Lowest (T1) HR (95%CI)	Middle (T2) HR (95%CI)	Highest (T3) HR (95%CI)	
Postmenopause					
No. cases	107	45	37	25	
Multivariate-adjusted ^a		1.00 (Reference)	0.90 (0.58-1.40)	0.69 (0.41-1.14)	.15

^aMultivariate-adjusted model 1, adjusted for age (years), area (10 public health center areas), body mass index (<25, ≥25 kg/m², or missing), age at menarche (≤13, 14, 15, ≥16 years, or missing), age at first delivery (<26, ≥26 years, or missing), number of deliveries (0, 1-2, 3, ≥4, or missing), menopause status and age at menopause (premenopause, postmenopause [<49, 50-54, ≥55 years], or missing), use of exogenous female hormones (yes, no, or missing), smoking status (current or ever, never, or missing), and alcohol intake (<150 or ≥150 g/week).

^bMultivariate-adjusted model 2 was further adjusted for energy-adjusted coffee intake (continuous) in addition to the variables in model 1.

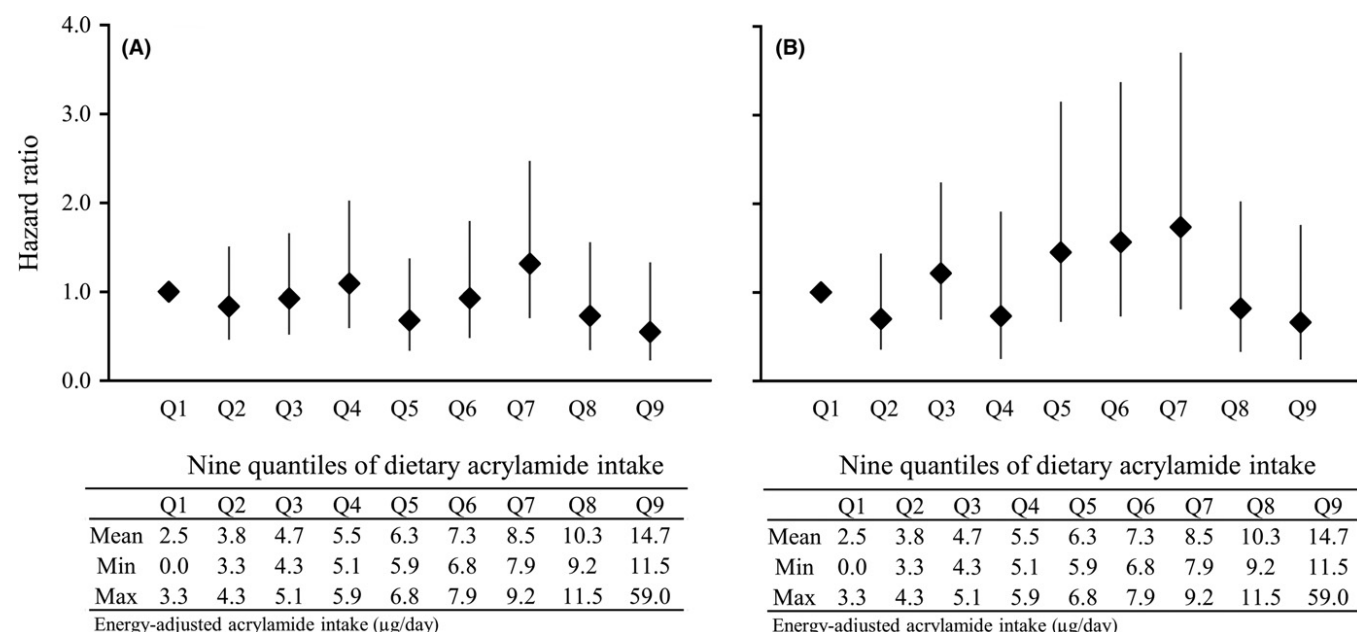


FIGURE 2 Hazard ratio (HR) for A, endometrial cancer risk and B, ovarian cancer risk vs 9 quantiles of dietary acrylamide intake. The reference group was the lowest ninth quantile of energy-adjusted dietary acrylamide intake. A, HR and 95%CI were adjusted for age (years), area (10 public health center areas), body mass index (<25, ≥25 kg/m², or missing), age at menarche (≤13, 14, 15, ≥16 years, or missing), age at first delivery (<26, ≥26 years, or missing), number of deliveries (0, 1-2, 3, ≥4, or missing), menopause status and age at menopause (premenopause, postmenopause [<49, 50-54, ≥55 years], or missing), use of exogenous female hormones (yes, no, or missing), smoking status (current, ever, never, or missing), alcohol intake (<150 or ≥150 g/week), and energy-adjusted coffee intake (continuous). Number of cases from the lowest to the highest of the nine quantiles was 25, 20, 22, 21, 13, 17, 23, 12, and 8, respectively. B, HR and 95%CI were adjusted for age (years), area (10 public health center areas), body mass index (<25, ≥25 kg/m², or missing), age at menarche (≤14, ≥15 years, or missing), age at first delivery (<26, ≥26 years, or missing), number of deliveries (0, 1-2, ≥3, or missing), menopause status (premenopause, postmenopause, or missing), use of exogenous female hormones (yes, no, or missing), smoking status (current or ever, never, or missing), and alcohol intake (<150 or ≥150 g/week). Number of cases from the lowest to the highest of the nine quantiles was 16, 11, 19, 8, 16, 17, 19, 9, and 7, respectively.

Studies found null associations for endometrial and ovarian cancers.³⁸⁻⁴¹ Differences in the dietary acrylamide intake in these cohorts may be one of the reasons for the lack of association. The average acrylamide intakes in the lowest and highest quintiles were 9.5 and 36.8 µg/day in the NLCS, and 9 and 26 µg/day in the NHS, respectively.^{35,36} Likewise, the EPIC cohort, which included 10 European countries, also had a wide intake range: the lowest was 8.8 µg/day in Italy and the highest was 35.5 µg/day in Denmark.³⁷ In contrast to these cohorts, the range of intake was narrower in the studies that showed no association.³⁸⁻⁴¹ In the Japanese participants, intake range

of quantiles, as well as the average intake, was considerably smaller than in the other studies that showed a significant association.

Differences in the contributing foods also may have had an impact on the results. In this study, specifically, decreased risk was observed with endometrial cancer in model 1, but the association was attenuated after the adjustment for coffee consumption. Coffee intake was reported as a probable preventive factor for endometrial cancer in the World Cancer Research Fund International.¹⁴ This was consistent with the decreased risk of endometrial cancer due to coffee consumption in the JPHC Study.³⁴

TABLE 3 Acrylamide intake and the risk of ovarian cancer

		Tertiles of acrylamide intake			P for trend
		Total	Lowest (T1) HR (95%CI)	Middle (T2) HR (95%CI)	
All women					
No. participants	47 185	15 728	15 729	15 728	
No. cases	122	46	41	35	
Person-years	733 572	246 889	244 758	241 925	
Age- and area-adjusted		1.00 (Reference)	0.90 (0.59-1.38)	0.76 (0.48-1.21)	.26
Multivariate-adjusted ^a		1.00 (Reference)	0.90 (0.59-1.38)	0.77 (0.49-1.23)	.28
Multivariate-adjusted (excluding cases ≤ 3 y) ^a		1.00 (Reference)	0.83 (0.52-1.33)	0.69 (0.41-1.16)	.16
By smoking status					
Current or past smoker					
No. cases	4	2	1	1	
Multivariate-adjusted ^a		1.00 (Reference)	0.42 (0.03-5.14)	0.23 (0.02-3.39)	.27
Never smoker					
No. cases	111	41	38	32	
Multivariate-adjusted ^a		1.00 (Reference)	0.94 (0.60-1.48)	0.82 (0.50-1.33)	.43
By coffee consumption					
<1 cup/week					
No. cases	39	25	10	4	
Multivariate-adjusted ^a		1.00 (Reference)	0.90 (0.43-1.90)	0.62 (0.21-1.82)	.40
≥1 cup/week					
No. cases	83	21	31	31	
Multivariate-adjusted ^a		1.00 (Reference)	1.02 (0.58-1.79)	0.95 (0.53-1.71)	.86
By alcohol consumption					
<150 g/week					
No. cases	117	42	40	35	
Multivariate-adjusted ^a		1.00 (Reference)	0.95 (0.61-1.48)	0.84 (0.52-1.35)	.47
≥150 g/week					
No. cases	5	4	1	0	
Multivariate-adjusted ^a		1.00 (Reference)	0.74 (0.05-12.14)	–	.35
By body mass index					
<25 kg/m ²					
No. cases	89	31	33	25	
Multivariate-adjusted ^a		1.00 (Reference)	1.09 (0.66-1.80)	0.83 (0.48-1.45)	.53
≥25 kg/m ²					
No. cases	31	14	8	9	
Multivariate-adjusted ^a		1.00 (Reference)	0.54 (0.22-1.32)	0.64 (0.26-1.55)	.29
By menopause status					
Premenopause					
No. cases	25	8	9	8	
Multivariate-adjusted ^a		1.00 (Reference)	0.87 (0.33-2.27)	0.70 (0.25-1.92)	.48
Postmenopause					
No. cases	94	35	32	27	
Multivariate-adjusted ^a		1.00 (Reference)	0.96 (0.59-1.57)	0.86 (0.51-1.46)	.58

^aMultivariable Cox proportional hazard models were adjusted for age (years), area (10 public health center areas), body mass index (<25, ≥ 25 kg/m², or missing), age at menarche (≤ 14 , ≥ 15 y, or missing), age at first delivery (<26, ≥ 26 y, or missing), number of deliveries (0, 1-2, ≥ 3 , or missing), menopausal status (premenopause, postmenopause, or missing), use of exogenous female hormones (yes, no, or missing), smoking status (current or ever, never, or missing), and alcohol intake (<150 or ≥ 150 g/week).

In the present study, as dietary acrylamide intake increased, the proportion of acrylamide intake from coffee was increased. It was thought that the significantly decreased risk observed in model 1 might be due to the preventive effect of coffee intake. Therefore, the beneficial effect of coffee intake may be greater than the influence of acrylamide intake accompanying coffee consumption, given that coffee was the highest contributing food to total acrylamide intake in this study. In the NHS, coffee was also one of the contributing foods, but, the risk of endometrial cancer increased.³⁶ This difference may be due to the high acrylamide intake and the difference in contributing foods other than coffee.

This study has several limitations. First, the associations might have been attenuated by FFQ measurement errors. Second, the results may be affected by residual confounding factors such as passive smoking. Given, however, that there were no questions regarding passive smoking in the 5-year follow-up FFQ, we could not include passive smoking in the adjusted model in the present study. To eliminate these effects, therefore, further studies using biomarkers are needed. Also, of the risk assessments on endometrial cancer, the FFQ result is inconsistent with the results using biomarkers such as hemoglobin adduct concentration in blood.^{6,37} Third, the assessment of acrylamide intake was done once. Therefore, we could not consider individual dietary changes during the follow-up period. There may be differences in the contributing food groups by generation, given that the proportion of acrylamide intake from beverages and from vegetables was lower and higher, respectively, in the Japanese 2012 national dietary survey estimations compared with the present results in the 1990s.²² Individual dietary habits, however, might not have changed dramatically, because the present participants were aged 45–74 years and their dietary habits were considered to be well established. Fourth, the low cancer incidence might affect the statistical power. The number of endometrial and of ovarian cancer cases was low in this cohort, reflecting the low incidence rate in Japan: age-standardized rates per 100 000 population in 2012 in Japan were 10.6 for endometrial cancer and 8.4 for ovarian cancer.⁴²

The strengths of this study were that the JPHC Study is one of the largest prospective cohort studies on lifestyle diseases; and recall bias on the exposure was prevented because the data were collected before cancer diagnosis. Participants were selected from the general population and the survey response rate was >80% while the loss to follow-up rate was considerably small. The proportion of DCO was <10% each for endometrial and ovarian cancers. Therefore, the follow-up data and the cancer registry in this study population were of sufficient quality.

This is the first study to assess the effect of dietary acrylamide intake on endometrial or ovarian cancer risks in Asian countries. We found no association between dietary acrylamide intake and endometrial or ovarian cancer risks regardless of smoking status, coffee consumption, alcohol consumption, body size, or menopause status in this large prospective cohort study of Japanese women with a relatively lower dietary intake of acrylamide compared with Western populations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

J.I. and T.S. designed the research; S.T., T.S., J.I., N.S., and M.I. conducted the research; A.K. contributed to the calculation of dietary acrylamide intake; A.K., L.Z., and R.L. performed the statistical analysis; A.K. interpreted the results and wrote the paper; and J.I. was primarily responsible for the final content. All authors reviewed the manuscript and contributed to the discussion.

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REFERENCES

1. International Agency for Research on Cancer. IARC working group on the evaluation of carcinogenic risks to humans: some industrial chemicals. *IARC Monogr Eval Carcinog Risks Hum*. 1994;60:1-560.
2. Food Safety Commission of Japan. Evaluation document of dietary acrylamide produced by heating. [Food Safety Commission of Japan.] 2016. https://www.fsc.go.jp/osirase/acrylamide1.data/acrylamide_hyokasyo1.pdf. Accessed January 17, 2017.
3. Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem*. 2002;50:4998-5006.
4. Pelucchi C, Bosetti C, Galeone C, La Vecchia C. Dietary acrylamide and cancer risk: an updated meta-analysis. *Int J Cancer*. 2015;136:2912-2922.
5. Shipp A, Lawrence G, Gentry R, et al. Acrylamide: review of toxicity data and dose-response analyses for cancer and noncancer effects. *Crit Rev Toxicol*. 2006;36:481-608.
6. Obón-Santacana M, Freisling H, Peeters PH, et al. Acrylamide and glycidamide hemoglobin adduct levels and endometrial cancer risk: a nested case-control study in nonsmoking postmenopausal women from the EPIC cohort. *Int J Cancer*. 2016;138:1129-1138.
7. Obón-Santacana M, Lujan-Barroso L, Travis RC, et al. Acrylamide and glycidamide hemoglobin adducts and epithelial ovarian cancer: a nested case-control study in nonsmoking postmenopausal women from the EPIC cohort. *Cancer Epidemiol Biomarkers Prev*. 2016;25:127-134.

8. Hogervorst JG, Fortner RT, Mucci LA, et al. Associations between dietary acrylamide intake and plasma sex hormone levels. *Cancer Epidemiol Biomarkers Prev.* 2013;22:2024-2036.
9. Nagata C, Konishi K, Tamura T, et al. Associations of acrylamide intake with circulating levels of sex hormones and prolactin in premenopausal Japanese women. *Cancer Epidemiol Biomarkers Prev.* 2015;24:249-254.
11. Granby K, Nielsen NJ, Hedegaard RV, Christensen T, Kann M, Skibsted LH. Acrylamide-asparagine relationship in baked/toasted wheat and rye breads. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2008;25:921-929.
12. Konings EJ, Hogervorst JG, van Rooij L, et al. Validation of a database on acrylamide for use in epidemiological studies. *Eur J Clin Nutr.* 2010;64:534-540.
13. Freisling H, Moskal A, Ferrari P, et al. Dietary acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition differs greatly according to geographical region. *Eur J Nutr.* 2013;52:1369-1380.
14. World Cancer Research Fund International. Summary of strong evidence on diet, nutrition, physical activity and the prevention of cancer. [World Cancer Research Fund International.] <https://www.wcrf.org/int/research-we-fund/continuous-update-project-findings-reports/endometrial-womb-cancer>. Accessed August 15, 2017.
15. Kotemori A, Ishihara J, Zha L, et al. Dietary acrylamide intake and risk of breast cancer: the Japan Public Health Center-based Prospective Study. *Cancer Sci.* 2018;109:843-853.
16. Watanabe S, Tsugane S, Sobue T, Konishi M, Baba S. Study design and organization of the JPHC study. Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases. *J Epidemiol.* 2001;11:S3-S7.
17. Tsugane S, Sawada N. The JPHC study: design and some findings on the typical Japanese diet. *Jpn J Clin Oncol.* 2014;44:777-782.
18. Resources Council, Science and Technology Agency, Government of Japan. *Standard Tables of Food Composition in Japan*, 5th edn. Tokyo: Printing Bureau, Ministry of Finance; 2002.
19. Ishihara J, Inoue M, Kobayashi M, et al. Impact of the revision of a nutrient database on the validity of a self-administered food frequency questionnaire (FFQ). *J Epidemiol.* 2006;16:107-116.
20. Tsugane S, Sasaki S, Kobayashi M, Tsubono Y, Akabane M; JPHC. Validity and reproducibility of the self-administered food frequency questionnaire in the JPHC Study cohort I: study design, conduct and participant profiles. *J Epidemiol.* 2003;13(1 Suppl):S2-S12.
21. Ishihara J, Sobue T, Yamamoto S, et al. Validity and reproducibility of a self-administered food frequency questionnaire in the JPHC study cohort II: study design, participant profile and results in comparison with cohort I. *J Epidemiol.* 2003;13(1 Suppl):S134-S147.
22. Food Safety Commission of Japan. Study on estimate of acrylamide intake from food; interim report. 2016. <https://www.fsc.go.jp/fscis/technicalResearch/show/cho99920151507>. Accessed January 17, 2017.
23. National Institute for Environmental Studies, Japan. Study on statistical estimate of acrylamide intake from foods. [National Institute for Environmental Studies, Japan.] <http://www.fsc.go.jp/fscis/technicalResearch/show/cho99920141408>. Accessed January 17, 2017.
24. Ministry of Agriculture, Forestry and Fisheries. Risk profile sheet relating to the food safety; for acrylamide. [Ministry of Agriculture, Forestry and Fisheries.] http://www.maff.go.jp/j/syouan/seisaku/risk_analysis/priority/pdf/150807_rp_aa.pdf. Accessed January 17, 2017.
25. National Institute of Health Sciences. Acrylamide analysis in food. [National Institute of Health Sciences.] <http://www.mhlw.go.jp/topic/s/2002/11/tp1101-1a.html>. Accessed January 17, 2017.
26. Mizukami Y, Kohata K, Yamaguchi Y, et al. Analysis of acrylamide in green tea by gas chromatography-mass spectrometry. *J Agric Food Chem.* 2006;54:7370-7377.
27. Takatsuki S, Nemoto S, Sasaki K, Maitani T. [Production of acrylamide in agricultural products by cooking.] *Shokuhin Eiseigaku Zasshi.* 2004;45:44-48 (in Japanese).
28. Yoshida M, Ono H, Ohnishi-Kameyama M, et al. [Determination of acrylamide in processed foodstuffs in Japan.] *Nippon Shokuhin Kagaku Kogaku Kaishi.* 2002;49:822-825 (in Japanese).
29. Yoshida M, Miyoshi K, Horibata K, Mizukami Y, Takenaka M, Yasui A. [Estimation of acrylamide intake from cooked rice in Japan.] *Nippon Shokuhin Kagaku Kogaku Kaishi.* 2011;58:525-530 (in Japanese).
30. Food Safety Commission of Japan. Information clearing sheet for acrylamide. [Food Safety Commission of Japan.] <https://www.fsc.go.jp/fscis/attachedFile/download?retrievalId=kai20111222sfc&fileId=520>. Accessed January 17, 2017.
31. Food and Agriculture Organization/World Health Organization. Health implications of acrylamide in food. 2002. <http://www.who.int/foodsafety/publications/acrylamide-food/en/> Accessed January 17, 2017.
32. International Agency for Research on Cancer. *Cancer Incidence in Five Continents*. Vol 9. Lyon, France: IARC Scientific Publications; 2008.
33. Weiderpass E, Sandin S, Inoue M, et al. Risk factors for epithelial ovarian cancer in Japan: results from the Japan Public Health Center-based Prospective Study cohort. *Int J Oncol.* 2012;40:21-30.
34. Shimazu T, Inoue M, Sasazuki S, et al. Coffee consumption and risk of endometrial cancer: a prospective study in Japan. *Int J Cancer.* 2008;123:2406-2410.
35. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev.* 2007;16:2304-2313.
36. Wilson KM, Mucci LA, Rosner BA, Willett WC. A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancers. *Cancer Epidemiol Biomarkers Prev.* 2010;19:2503-2515.
37. Obon-Santacana M, Kaaks R, Slimani N, et al. Dietary intake of acrylamide and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort. *Br J Cancer.* 2014;111:987-997.
38. Larsson SC, Hakansson N, Akesson A, Wolk A. Long-term dietary acrylamide intake and risk of endometrial cancer in a prospective cohort of Swedish women. *Int J Cancer.* 2009;124:1196-1199.
39. Larsson SC, Akesson A, Wolk A. Long-term dietary acrylamide intake and risk of epithelial ovarian cancer in a prospective cohort of Swedish women. *Cancer Epidemiol Biomarkers Prev.* 2009;18:994-997.
40. Pelucchi C, Galeone C, Negri E, et al. Dietary acrylamide and the risk of endometrial cancer: an Italian case-control. *Nutr Cancer.* 2016;68:187-192.
41. Pelucchi C, Galeone C, Levi F, et al. Dietary acrylamide and human cancer. *Int J Cancer.* 2006;118:467-471.
42. Ervik M, Lam F, Ferlay J, Mery L, Soerjomataram I, Bray F. *Cancer Today*. Lyon, France: International Agency for Research on Cancer; 2016.

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A prospective cohort study on dietary acrylamide intake and the risk for cutaneous malignant melanoma

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Epidemiological studies have shown inconsistent associations between dietary acrylamide exposure and the risk for various malignancies. This is the first epidemiological study on the association between acrylamide intake and the risk for cutaneous malignant melanoma (CMM). A case-cohort analysis was carried out within the prospective Netherlands Cohort Study on diet and cancer. Acrylamide intake was estimated from a food frequency questionnaire combined with acrylamide data for Dutch foods. After 17.3 years of follow-up, 501 microscopically confirmed cases of CMM were identified. There was an increased risk for CMM when dietary acrylamide was modeled as a continuous variable [hazard ratio: 1.13 (95% confidence interval: 1.01–1.26)] per 10 µg increment among men but there was no clear linear trend over the quintiles ($P_{\text{trend}} = 0.12$). No associations were observed for women. Our study provides some indications

that dietary acrylamide may increase the risk for CMM in men. *European Journal of Cancer Prevention* 26:528–531 Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.

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Keywords: cutaneous malignant melanoma, dietary acrylamide, prospective cohort study

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Introduction

Acrylamide is classified as a probable human carcinogen based on its observed carcinogenicity in rodent studies (Pelucchi *et al.*, 2015). Acrylamide forms in heat-treated starchy foods, such as coffee, French fries, and cookies (Pelucchi *et al.*, 2015).

Results from epidemiological studies indicate that dietary acrylamide might be associated with the risk for kidney, endometrial, and ovarian cancers but the results were inconsistent (Pelucchi *et al.*, 2015). Every tissue is a potential target for acrylamide-induced carcinogenesis because acrylamide is distributed throughout the whole body.

Our objective was to investigate, for the first time, the association between dietary acrylamide intake and the risk for cutaneous malignant melanoma (CMM).

Materials and methods

The association between acrylamide intake and CMM risk was investigated in the Netherlands Cohort Study on diet and cancer (NLCS) (van den Brandt *et al.*, 1990), a case-cohort study. CMM cases from the whole cohort were identified during follow-up, and a random sample of 5000 men and women sampled at baseline served as a subcohort from which accumulated person-years for the

entire cohort (120 852 participants) were estimated. There were 1951 male subcohort members and 224 male CMM cases, and 2101 female subcohort members and 224 female CMM cases available for analysis (Supplemental Fig. 1, Supplemental digital content 1, <http://links.lww.com/EJCP/A66>).

Histologically confirmed CMM cases were identified through linkage with the Dutch Pathology Registry (PALGA) and the National Dutch Cancer Registry. Completeness of follow-up of these registries is estimated to be at least 96% (Schouten *et al.*, 1993). Follow-up for vital status in the NLCS, as assessed through linkage with the Municipal Personal Records Database (GBA), at the end of the follow-up period (17.3 years) was nearly 100%; only one male subcohort member was lost to follow-up.

The NLCS has been approved by the Medical Ethics Committee of Maastricht University (Maastricht, the Netherlands).

Acrylamide intake was assessed using a self-administered food frequency questionnaire (FFQ) on 150 food items, and from this FFQ acrylamide intake was estimated as described elsewhere (Hogervorst *et al.*, 2007).

The NLCS questionnaire did not contain direct questions on UV exposure. To adjust for nonoccupational UV exposure, proxies using open-ended questions on hobbies and sports were constructed. The women's version of the NLCS

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questionnaire did not contain a question concerning hobbies, and hence for women we only constructed a proxy based on sports. A maximum of three hobbies and sports could be entered by the participants. Three variables were created for UV exposure through hobbies and three similar variables for UV exposure through sports: exposure to UV of limbs only (men and women combined) and exposure to UV of both limbs and trunk (sex-specific). Variables were coded as 1 (exposure to UV likely) and 0 (exposure to UV unlikely) (Supplemental Table 1, Supplemental digital content 2, <http://links.lww.com/EJCP/A67> and 2, Supplemental digital content 3, <http://links.lww.com/EJCP/A68>). Coding was carried out independently by two researchers (N.L. and J.G.F.H.). Divergent codings were discussed until consensus was reached. To adjust for occupational UV exposure, information from the Finnish job-exposure matrix (FINJEM) was used (Kauppinen *et al.*, 2009). Exposure estimates in FINJEM are provided for the period between 1960 and 2003 and are presented as a proportion of workers exposed (*P*) multiplied by the level of exposure (*L*). Job codes assigned in the NLCS based on data on occupation were translated into those compatible with FINJEM (Koeman *et al.*, 2013).

Hazard ratios (HR) were obtained using Cox proportional hazards models for men and women separately. SEs were

estimated using the robust Huber–White sandwich estimator. The proportional hazards assumption was tested using scaled Schoenfeld residuals. HRs were adjusted for covariables: age, smoking status, smoking frequency and duration, body mass index, and education level. The proxies for UV exposure did not change the HRs of acrylamide and were therefore not included in the models.

Subgroup analyses were carried out separately for histological subtypes [nodular (NM) and superficial spreading melanoma (SSM)] and for never-smokers (or for men: never-smokers and those who quit smoking at least 10 years before baseline) because smoking causes substantial acrylamide exposure (Hagmar *et al.*, 2005).

A result was considered statistically significant if the *P*-value was 0.05 or less (two sided). STATA software (StataCorp 2011, Stata Statistical Software: Release 12; StataCorp LP, College Station, Texas, USA) was used for all statistical analyses.

Results

For most variables there were no striking differences between CMM, NM, and SSM cases and subcohort members (Table 1). However, cases were more highly

Table 1 Characteristics of total cutaneous malignant, superficial spreading, and nodular melanoma cases and subcohort members: the Netherlands cohort study on diet and cancer, 1986–2003^{a,b}

	Men				Women			
	Subcohort	CMM	SSM	NM	Subcohort	CMM	SSM	NM
<i>N</i>	2191	241	94	40	2247	236	102	30
Acrylamide (μg/day)	22.6 (12.2)	23.9 (13)	24.1 (12.4)	27.5 (15.4)	21.1 (11.9)	21.2 (11.7)	20.5 (10.9)	21.5 (12.6)
Age (years)	61.3 (4.2)	61.7 (4.3)	61.2 (4.3)	62.8 (4.5)	61.4 (4.3)	61.9 (4.1)	61.6 (4.1)	61.4 (4.2)
BMI (kg/m ²)	25 (2.6)	25.4 (2.7)	25.3 (3)	25.1 (2.1)	25.1 (3.6)	24.8 (3.4)	24.5 (3.1)	25.0 (4.3)
Smoking status (%)								
Never-smokers	12.7	17.8	20.2	12.5	58.4	54.2	53.9	50
Former smokers	51.6	59.8	59.6	65	20.6	28	32.4	26.7
Current smokers	35.7	22.4	20.2	22.5	21	17.8	13.7	23.3
Smoking [years (former and current smokers only)]	33.7 (11.8)	29.8 (13)	28.4 (13.8)	30.9 (12.7)	27.8 (12.5)	26.8 (12.4)	25.7 (12.4)	25.7 (13)
Smoking [cigarettes/day (former and current smokers only)]	17 (10.6)	15 (10.5)	14.4 (9.3)	17.2 (12.1)	11.4 (8.3)	12.2 (11.2)	12.7 (9.5)	19.2 (20.2)
Education (%)								
Primary school	25	16.6	13.8	15	33.5	27.7	26.7	23.3
Lower vocational school	20.7	13.7	11.7	7.5	23.2	23.4	25.7	16.7
Intermediate vocational school/high school	35.6	4.7	42.6	50	34.5	37	36.6	46.7
Higher vocational school/university	18.7	29.1	31.9	27.5	8.8	11.9	10.9	13.3
Occupational UV exposure (%)								
Never	72.9	78.0	77.7	80.0	97	95.8	97.1	90
Ever	12.8	10.4	11.7	10.0	1.5	2.5	0.0	0.0
High	14.3	11.6	10.6	10.0	1.5	1.7	2.9	10.0
Cumulative exposure (<i>P</i> × <i>L</i> /100)	761 (1951)	673 (1770)	697 (1708)	434 (1234)	57.3 (472)	80.1 (589)	126 (820)	36.0 (121)
Nonoccupational UV exposure to the limbs from (%)								
Hobbies (yes)	41.5	36.5	35.1	42.5	^c	^c	^c	^c
Sports (yes)	45.1	49.8	45.4	55.0	23.1	27.5	26.5	40.0
Nonoccupational UV exposure to the limbs and trunk from (%)								
Hobbies (yes)	30.8	27.8	29.8	22.5	^c	^c	^c	^c
Sports (yes)	16.0	18.3	18.1	20.0	16.6	22.5	20.6	30.0

P, prevalence of exposure (%); *L*, level of exposure (J/m²) (Kauppinen *et al.*, 2009).

CMM, cutaneous malignant melanoma; NM, nodular melanoma; NLCS, the Netherlands cohort study on diet and cancer; SSM, superficial spreading melanoma.

^aData represent means (SD), or percentages unless otherwise indicated; *n* represents number of subcohort members or cases after exclusion of participants with prevalent cancer at baseline and/or with incomplete or inconsistent dietary data.

^bThe number of missing values varies.

^cThe NLCS questionnaire contained a question on hobbies for men only.

Table 2 Association between dietary acrylamide intake and cutaneous malignant melanoma in men and women: the Netherlands cohort study on diet and cancer, 1986–2003

	Overall				Never-cigarette-smokers and 10-year quitters of cigarette smoking			
	N (cases)	N (person years)	HR (95% CI) ^a	HR (95% CI) ^b	N (cases)	N (person years)	HR (95% CI) ^a	HR (95% CI) ^c
Men								
Total cutaneous malignant melanoma								
AA (10 µg/day)	224	28 124	1.10 (0.99–1.22)	1.13 (1.01–1.26)	136	13 340	1.04 (0.90–1.20)	1.07 (0.92–1.26)
Q1	45	5625	Reference	Reference	32	2870	Reference	Reference
Q2	46	5435	1.08 (0.70–1.67)	1.14 (0.73–1.80)	28	2675	0.96 (0.55–1.67)	0.99 (0.55–1.77)
Q3	35	5734	0.81 (0.51–1.30)	0.90 (0.55–1.45)	21	2383	0.82 (0.45–1.50)	0.85 (0.46–1.58)
Q4	38	5609	0.91 (0.57–1.44)	1.03 (0.64–1.66)	22	2482	0.83 (0.46–1.49)	0.89 (0.48–1.64)
Q5	60	5721	1.37 (0.90–2.07)	1.52 (0.98–2.33)	33	2930	1.03 (0.60–1.77)	1.13 (0.63–2.00)
<i>P</i> _{trend} ^d			0.29	0.12			0.94	0.81
Nodular melanoma								
AA (10 µg/day)	39	28 124	1.27 (1.07–1.51)	1.36 (1.11–1.67)	25	13 340	1.37 (1.11–1.69)	1.60 (1.19–2.15)
Superficial spreading melanoma								
AA (10 µg/day)	86	28 124	1.10 (0.94–1.28)	1.14 (0.97–1.34)	54	13 340	0.92 (0.74–1.13)	0.94 (0.75–1.18)
Q1	14	5877	Reference	Reference	20	4122	–	–
Q2	23	6829	0.96 (0.51–1.81)	1.02 (0.53–1.95)	19	4457	–	–
Q3	23	8228	1.10 (0.59–2.05)	1.26 (0.66–2.40)	15	4762	–	–
Q4	26	7189	1.26 (0.69–2.31)	1.42 (0.77–2.64) ^f	–	–	–	–
<i>P</i> _{trend} ^d			0.40	0.21				
Women								
Total cutaneous malignant melanoma								
AA (10 µg/day)	224	32 990	0.97 (0.87–1.09)	0.97 (0.86–1.08)	123	19 939	1.03 (0.88–1.20)	1.02 (0.88–1.20)
Q1	43	6433	Reference	Reference	21	4238	Reference	Reference
Q2	47	6695	1.08 (0.70–1.67)	1.10 (0.71–1.72)	27	4060	1.37 (0.75–2.48)	1.39 (0.76–2.56)
Q3	48	6354	1.19 (0.77–1.84)	1.25 (0.80–1.96)	28	3561	1.74 (0.96–3.15)	1.78 (0.96–3.30)
Q4	47	6857	1.07 (0.69–1.66)	1.11 (0.71–1.73)	25	4107	1.30 (0.71–2.39)	1.31 (0.71–2.43)
Q5	39	6650	0.91 (0.58–1.43)	0.91 (0.57–1.44)	22	3972	1.16 (0.62–2.15)	1.16 (0.62–2.19)
<i>P</i> _{trend} ^d			0.70	0.71			0.72	0.74
Nodular melanoma								
AA (10 µg/day)	29	32 990	0.94 (0.68–1.29)	0.92 (0.68–1.25)	14	19 939	–	–
Superficial spreading melanoma								
AA (10 µg/day)	94	32 990	0.92 (0.77–1.10)	0.91 (0.76–1.08)	52	19 939	0.95 (0.76–1.19)	0.94 (0.75–1.19)
Q1	26	9424	Reference	Reference	22	7828	–	–
Q2	28	8405	1.25 (0.72–2.20)	1.30 (0.73–2.31)	18	5816	–	–
Q3	23	7077	1.11 (0.62–1.99)	1.16 (0.64–2.09)	12	6295	–	–
Q4	17	8084	0.73 (0.39–1.37)	0.71 (0.37–1.35)	–	–	–	–
<i>P</i> _{trend} ^d			0.27	0.25				

The median acrylamide intake of the male subcohort in the quintiles was 10.8, 15.6, 19.6, 25.4, and 37.6 µg/day. The median acrylamide intake of the female subcohort in the quintiles was 9.5, 14.0, 17.9, 24.3, and 36.8 µg/day.

–, Too few cases to carry out the analysis; 95% CI, 95% confidence interval; HR, hazard ratio.

^aAdjusted for age.

^bAdjusted for age, education level (primary school, lower vocational school, intermediate vocational/high school, higher vocational school/university), BMI (kg/m²), cigarette smoking status (never/former/current smokers), number of cigarettes smoked per day, number of years of smoking cigarettes.

^cAdjusted for age, education level (primary school, lower vocational school, intermediate vocational/high school, higher vocational school/university), BMI (kg/m²), number of cigarettes smoked per day, number of years of smoking cigarettes.

^dThe *P*-value for trend was calculated by modeling the median acrylamide intake value in each quantile as a continuous variable.

^eAdjusted for age, education level (primary school, lower vocational school, intermediate vocational/high school, higher vocational school/university), BMI (kg/m²).

^fProportional hazards assumption was violated.

educated than subcohort members, whereas smoking was more prevalent among subcohort members.

There was a statistically significant positive multivariable-adjusted association between acrylamide intake modeled per 10 µg/day increment and total CMM risk among men [HR 1.13 (95% CI: 1.01–1.26)] (Table 2). The quintile analysis, however, did not reflect a linear dose–response relationship (*P*_{trend}: 0.12). The association was less strong in nonsmoking men, with an HR of 1.07 (95% CI: 0.92–1.26) per 10 µg acrylamide increment.

Subtype analyses showed a statistically significant positive association between dietary acrylamide and the risk for NM among men [HR 1.36 (95% CI: 1.11–1.67)] per 10 µg acrylamide increment (Table 2). A stronger association was seen among nonsmoking men [HR 1.60 (95% CI: 1.19–2.15)]. No statistically significant associations were observed between dietary acrylamide intake and the risk for SSM among men. The proportional hazards assumption was violated in the highest quartile of dietary acrylamide intake in the analysis of SMM but no statistically significant interaction with time was observed.

Among women, there was no association between acrylamide intake and melanoma risk (Table 2).

Discussion

This study provides some indications that dietary acrylamide may increase the risk for overall CMM and NM among men. There was no positive association with total CMM risk in the group of nonsmoking men. For NM, however, the association between dietary acrylamide intake and NM risk was stronger in nonsmoking men. The latter finding may be spurious because of the small number of cases. No association was observed for SSM. No statistically significant associations were observed among women.

Differences in biological mechanisms in the etiology of different histological CMM subtypes are still unclear (Whiteman *et al.*, 2011) but it is possible that acrylamide has a differential effect on different subtypes. Acrylamide has been previously shown to cause genotoxicity and to influence sex hormone levels in rodents (Besaratina and Pfeifer, 2007). Although melanoma is not clearly a sex hormone-dependent cancer, it is likely that sex hormones are of importance in melanomagenesis (de Giorgi *et al.*, 2011) and their role may differ in men and women. Smoking influences sex hormone levels, which may explain the differential associations between acrylamide intake and melanoma risk based on smoking status.

Our study has a few limitations. FFQs have limitations with regard to assessing dietary acrylamide exposure (Hogervorst *et al.*, 2007). Nondifferential measurement error of the acrylamide intake resulting from the use of an FFQ in a prospective cohort study will push the risk estimate toward null. In addition, subgroup analyses were carried out with relatively small numbers. Therefore, the results have to be interpreted with caution. Proxies of sun exposure to the trunk and lower limbs due to hobbies and sports or occupational UV exposure were no confounders of the association between dietary acrylamide and the risk for CMM. In addition, the observation of similar risk estimates for different anatomical locations of CMM (results not shown) also suggests that UV exposure is not a confounder because anatomical location is a surrogate for assessing sun exposure patterns. All in all, it is unlikely that UV exposure was a confounder in this analysis but residual confounding cannot be ruled out.

Important strengths of the study are its prospective nature, large study size, and virtually no loss to follow-up.

In conclusion, our study gives some indications for a positive association between dietary acrylamide and the risk for total CMM and NM among men and no association among women.

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Conflicts of interest

There are no conflicts of interest.

References

- Besaratinia A, Pfeifer GP (2007). A review of mechanisms of acrylamide carcinogenicity. *Carcinogenesis* **28**:519–528.
- de Giorgi V, Sestini S, Gori A, Mazzotta C, Grazzini M, Rossari S, *et al.* (2011). Polymorphisms of estrogen receptors: risk factors for invasive melanoma – a prospective study. *Oncology* **80** (3–4):232–237.
- Hagmar L, Wirfalt E, Paulsson B, Tornqvist M (2005). Differences in hemoglobin adduct levels of acrylamide in the general population with respect to dietary intake, smoking habits and gender. *Mutat Res* **580** (1–2):157–165.
- Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2007). A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev* **16**:2304–2313.
- Kauppinen T, Heikkilä P, Plato N, Wlodbaek T, Lenvik K, Hansen J, *et al.* (2009). Construction of job-exposure matrices for the Nordic Occupational Cancer Study (NOCCA). *Acta Oncol* **48**:791–800.
- Koeman T, Offermans NS, Christopher-de Vries Y, Slottje P, van den Brandt PA, Alexandra Goldbohm R, *et al.* (2013). JEMs and incompatible occupational coding systems: effect of manual and automatic recoding of job codes on exposure assignment. *Ann Occup Hyg* **57**:107–114.
- Pelucchi C, Bosetti C, Galeone C, La Vecchia C (2015). Dietary acrylamide and cancer risk: an updated meta-analysis. *Int J Cancer* **136**:2912–2922.
- Schouten LJ, Hoppener P, van den Brandt PA, Kottner JA, Jager JJ (1993). Completeness of cancer registration in Limburg, the Netherlands. *Int J Epidemiol* **22**:369–376.
- van den Brandt PA, Goldbohm RA, van't Veer P, Volovics A, Hermus RJ, Sturmans F (1990). A large-scale prospective cohort study on diet and cancer in the Netherlands. *J Clin Epidemiol* **43**:285–295.
- Whiteman DC, Pavan WJ, Bastian BC (2011). The melanomas: a synthesis of epidemiological, clinical, histopathological, genetic, and biological aspects, supporting distinct subtypes, causal pathways, and cells of origin. *Pigment Cell Melanoma Res* **24**:879–897.



Dietary acrylamide exposure was associated with increased cancer mortality in Chinese elderly men and women: a 11-year prospective study of Mr. and Ms. OS Hong Kong

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Abstract

Aim Our study aims to investigate the association between dietary acrylamide exposure and cancer mortality among Chinese elderly.

Methods A prospective cohort of 4000 elderly men and women aged 65 years and above (Mr. and Ms. OS Hong Kong study) was recruited from local communities from 2001 to 2003. Dietary exposure to acrylamide was evaluated at baseline based on a validated food frequency questionnaire and an acrylamide database from the 1st Hong Kong Total Diet Study. Data on mortality statistics through March 2014 were obtained from the Death Registry of the Department of Health of Hong Kong with a median follow-up of 11.1 years. Cox proportional hazards models were used to examine the association between the acrylamide exposure and cancer mortality. Sex hormones were assessed in men.

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Results During a median follow-up of 11.1 years (39,271 person-years), we ascertained 330 cancer deaths. Vegetables (43.7%) and cereals (28.9%) products were the major contributors to dietary acrylamide. Compared with the lowest quartile of acrylamide intake (<9.9 µg/day), the multivariable hazard ratios for the highest quartile (>17.1 µg/day) were 1.9 (95% CI 1.3–2.8; $P_{\text{trend}} < 0.01$), 1.9 (95% CI 1.0–3.6; $P_{\text{trend}} = 0.05$), and 2.0 (95% CI 1.0–4.0; $P_{\text{trend}} = 0.06$) for the cancer mortality from overall, digestive and respiratory system, respectively. The associations were attenuated to null after further adjustment for circulating free estradiol in men. No statistically significant interactions were observed between acrylamide exposure and sex, obesity and overall lifestyle pattern scores.

Conclusions The longitudinal data provided evidence that dietary acrylamide, in amounts that Chinese elderly are typically exposed to, was associated with increased cancer mortality. Circulating free estradiol may mediate the association in men.

Keywords Dietary exposure · Acrylamide · Cancer mortality · Chinese elderly

Introduction

Cancer has become the most important cause of death in China and throughout the world (Stewart 2014). Acrylamide was classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC) on the basis of its carcinogenicity in rodents. Animal studies have shown acrylamide is a multisite carcinogen in rodents and causes tumors in skin, mesothelioma, lung, mammary gland, uterus, ovary, testis, thyroid, Harderian gland oral cavity and central nervous system (Bull et al. 1984; Johnson et al.

1986; Friedman 2003). A recent meta-analysis (Pelucchi et al. 2015) indicates that dietary acrylamide has a modest association with kidney cancer and with endometrial and ovarian cancer in never smokers.

Acrylamide is commonly present in carbohydrate-rich foods processed at high temperatures ($>120^{\circ}\text{C}$) and formed by Maillard browning reactions, in which amino acids, particularly asparagine, react with reducing sugars (Mottram et al. 2002). High levels of acrylamide have been found in fried and baked potato products and in cereal products such as crisp bread, breakfast cereals and cookies (Mottram et al. 2002). Due to its ubiquitous presence in foods with concentrations at considerably higher levels than other well-known food carcinogens (i.e., polycyclic aromatic hydrocarbons and ethyl carbamate); acrylamide might be responsible for a considerable part of the diet-related cancer incidences and mortality (JECFA 2005). Some studies suggest that acrylamide may affect the cancer risk through hormonal pathways (Hogervorst et al. 2013; Nagata et al. 2015).

Dietary acrylamide can be rapidly and extensively absorbed from the gastrointestinal tract, then metabolized and excreted in urine, mainly as mercapturic acid derivatives of acrylamide and glycidamide (GA). Many observational studies have focused on dietary acrylamide exposure and the incidences of individual cancers (Pelucchi et al. 2011). Few cohort studies have examined the association of acrylamide exposure and cancer mortality, especially among elderly populations. This study aimed to investigate the relationship of dietary acrylamide and cancer mortality, and explore whether endogenous sex hormones may mediate the association based on an 11-year follow-up of an elderly population in Hong Kong.

Methods

The study of Mr. and Ms. OS Hong Kong was originally designed to investigate the risk factors of osteoporosis. Elderly men and women aged 65 years and above were recruited from 2001 to 2003 through advertisements placed in community centers and housing estates. Those who were unable to walk without assistance of another person, had bilateral hip replacement or were not competent to give informed consent were excluded. The participants were stratified so that approximately 33% were in each of the age groups: 65–69, 70–74, and 75 and above. The study enrolled 4000 participants. Details of the study design have previously been published (Lau et al. 2006). The study was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong and complied with the Declaration of Helsinki. The informed consent for this study was obtained before study enrolment and during the data collection.

Eligible participants were invited to the Research Center for baseline clinical assessment, and face-to-face interviews

based on structured and standardized questionnaires. Information collected included social demographic data, family and medical history, current use of medications, smoking and drinking of alcohol, tea and coffee. Dietary intakes were assessed by a validated food frequency questionnaire (FFQ) based on the data from the Hong Kong Adult Dietary Survey (Woo et al. 1997). Physical activity was measured by the Physical Activity Scale for the Elderly Questionnaire (PASE) (Washburn et al. 1993). Height was measured by the Holtain Harpenden stadiometer (Holtain Ltd., Crosswell, UK). Body weight was measured by the Physician Beam Balance Scale (Healthometer, IL, USA).

A total of 1489 male subjects were randomly selected to have stored serum analyzed for sex hormones and their precursors (sex hormone-binding globulin (SHBG), estradiol, androstenedione, dehydroepiandrosterone, 5-androstene-3 β ,17 β -diol and dehydroepiandrosterone sulfate). Free fractions of estradiol were calculated as described by Sodergard et al. (1982). All hormone assays were performed by Gas chromatography–mass spectrometry (GC/MS) (Labrie et al. 2009).

Acrylamide intake assessment and the 1st total diet study in Hong Kong

The validated FFQ containing 329 food items was used to estimate dietary nutrients and acrylamide intakes at baseline (Woo et al. 1997). Acrylamide level in individual food items was based on the acrylamide database of the 1st Hong Kong Total Diet Study (HKTDS) (Centre for Food Safety and Department 2011). The food items in FFQ that were used in the acrylamide intake assessment were assigned the mean value of the acrylamide detected in the TDS food items or a value of one half the quantitation limit when concentrations were lower than the quantitation limit (Centre for Food Safety and Department 2011). Dietary acrylamide intake was estimated from the mean acrylamide level (Centre for Food Safety and Department 2011), the frequency of consumption and the portion size of the intake of food items. TDS has been recognized internationally as one of the most cost effective way to estimate dietary exposures to food chemicals for various population groups and to assess their associated health risks. Due to its nature of covering total diet, TDS can identify potentially contaminated foods or food groups that may be present at very low levels. In the 1st HKTDS, a total of 1800 samples for the 150 TDS food items were collected and prepared into table-ready forms on four occasions. On each occasion, three samples of each TDS food item were purchased from various retail outlets in different regions of the territory. The three samples of the same food item were then prepared as for normal consumption, i.e., table-ready, in a manner most representative of and consistent with cultural habits in Hong Kong as far as

practicable. They were then homogenized individually and combined into 600 composite samples for the laboratory analysis on food chemicals.

Case ascertainment and follow-up

Data on mortality statistics were obtained from the Death Registry of the Department of Health of Hong Kong till March 2014 with a median follow-up of 11.1 years. Cancer deaths were identified by the cause of death reported on the death certificate, and classified according to the International Classification of Disease (ICD) version 10 codes as those ranging from C00 to D48 for neoplasms. Follow-up of participants continued until death attributable to cancers, with censoring at the time of death for those who died from causes other than cancers.

Statistical analysis

Statistical analysis was conducted using SPSS version 21.0. All *P* values were two sided with significance level at 0.05. The contributions of 15 TDS food groups to overall dietary acrylamide exposure were calculated as percentages. We categorized participants into quartiles of dietary exposure of acrylamide based on its distribution in the entire cohort. Differences in the participants' characteristics by quartiles of dietary acrylamide exposure were compared using Chi-square test for categorical variables or analysis of variance (ANOVA) for continuous variables. Participants contributed person-time from baseline until the date of death, death from any cancers, or 31st March 2014, whichever occurred first. Participants with medical history of any cancers at baseline were excluded and 3823 remained for analysis. Cox proportional hazards models were used to estimate hazard risks (HR) with their 95% confidence intervals (CIs) for the association between acrylamide intake and overall cancer mortality, and cancer mortality from digestive tract and respiratory system, respectively. In multivariable analysis, we controlled for age, sex, education, body mass index (BMI) at baseline, physical activities, history of diabetes, cigarette smoking, total energy intake, alcohol, coffee and tea drinking, dietary carbohydrate and fibers. We tested the proportional hazard assumption using the likelihood ratio test and found no departure from the assumption. Tests for trend were conducted by assigning the median value of acrylamide exposure to each quartile and modeling this variable as a continuous variable. Because cigarette smoking is an important source of acrylamide exposure, we conducted a sensitivity analysis excluding the current smokers. To check for the influence of protopathic bias, the analyses were also done with exclusion of cancer deaths occurring in the first 2 years of follow-up.

Effect modification by other variables on the association between acrylamide intake and cancer mortality was tested. These variables in our analysis were sex, BMI, and score of AHA-DLR (the score of compliance of the guideline of American Heart Association Dietary and lifestyle Recommendation). To evaluate whether sex hormones mediated the association of acrylamide exposure with cancer mortality in men, we added sex hormones individually in the regression model. We tested for potential effect modification by including an interaction term of these variables with the quartiles of acrylamide exposure in the regression models. Subgroup analyses were further conducted.

Results

We observed a mean (\pm SD) daily intake of acrylamide of 14.6 ± 8.2 μ g/day in the study population. The main dietary sources of acrylamide (Table 1) were vegetables and their products (43.7%), followed by cereals and their products (28.9%), then fish, seafood and their products (8.93%), legumes, nuts and seeds and their products (6.75%) and mixed dishes (4.64%).

The participants' characteristics in quartiles of overall acrylamide intake were indicated in Table 2. Compared with the lowest acrylamide group, those in the highest quartile were younger, had higher education, body weight and smoking dosage; more likely to be married and physically active; higher AHA-DLR scores and higher intake of total energy, carbohydrate, protein and dietary fibers; had higher coffee, tea and alcohol intake; higher intake of French fries, fast foods and red/processed meat.

The crude and multivariable-adjusted associations (HR and 95% CI) between acrylamide exposure and cancer mortality are shown in Table 3. We ascertained 330 cancer deaths during total 39,271 person-years of follow-up (mean, 11.1 years). After adjustment for potential covariables, a significant association was observed between acrylamide intake and an overall increased cancer mortality with a HR of 1.9 (95% CI 1.3–2.8; $P_{\text{trend}} < 0.01$). Sensitivity analysis excluding current smokers ($n = 274$), or cases that died from cancer during the first 2 years of follow-up ($n = 61$) did not change the results materially. In the current non-smokers, the multivariable-adjusted HR in the highest quartile was 2.0 (95% CI 1.3–3.1; $P_{\text{trend}} < 0.01$) compared with the lowest quartile of acrylamide intake. Subgroup analyses for cancer mortality from digestive tract (HR 1.9; 95% CI 1.0–3.6; $P_{\text{trend}} = 0.05$) and respiratory system (HR 2.0; 95% CI 1.0–4.0; $P_{\text{trend}} = 0.06$) demonstrated similar HRs to those of overall mortality.

Table 4 indicated the results of subgroup analyses between acrylamide intake and potential variables of effect modification. There were no significant interactions between

Table 1 Dietary exposure ($\mu\text{g/day}$) to acrylamide and its contributions by food groups of total diet study (TDS)

TDS food groups	Dietary exposure ($\mu\text{g/day}$) Mean \pm standard deviation	% Contribution of total exposure
Cereals and their products	3.82 ± 1.68	28.90
Baked cereals and their products	2.67 ± 2.47	20.84
Vegetables and their products	6.52 ± 4.78	43.70
Stir-fried vegetables and their products	4.45 ± 5.16	29.65
Potatoes	0.19 ± 0.289	1.30
Legumes, nuts and seeds and their products	1.02 ± 1.40	6.75
Fruits	0.36 ± 0.29	2.65
Meat, poultry and game and their products	0.15 ± 0.20	1.07
Eggs and their products	0.01 ± 0.02	0.07
Fish, seafood and their products	1.58 ± 4.58	8.93
Dairy products	0.04 ± 0.08	0.27
Fats and oils	0.01 ± 0.08	0.19
Beverages, alcoholic	0.02 ± 0.01	0.07
Beverages, non-alcoholic	0.25 ± 0.32	1.86
Coffee	0.13 ± 0.38	0.94
Mixed dishes	0.63 ± 0.51	4.64
Snack foods	0.13 ± 0.59	0.74
Potato crisps	0.044 ± 0.079	0.30
Sugars and confectionery	0.02 ± 0.12	0.12
Condiments, sauces and herbs	0.00 ± 0.01	0.01
Total	14.57 ± 8.15	100

Half of limit of detection (LOD) is used for all results less than LOD in calculating the exposure estimates. Baked cereals included: pasta, instant noodles, bread, bun, biscuit, cakes, pastries, breakfast cereals and deep-fried dough. Fried vegetables and their products included 22 kinds of vegetables such as zucchini, eggplant, sweet pepper, tomato, garlic, onion, spring onion, mung bean sprout, spinach, and water spinach

TDS total diet study

quartiles of acrylamide intake and any of the variables on overall cancer mortality. The associations were largely consistent across sex, BMI (<24 and ≥ 24 kg/m^2) and AHA-DRL scores (above and below median). In men, after adjustment for circulating estradiol but not other sex hormones, the HRs were reduced to around 1, suggesting that the effect of acrylamide exposure on cancer mortality may be mediated through circulating free estradiol. When the analyses were further restricted to the subgroup of elderly who had never smoked, the associations (HRs) were similar but were marginally significant due to reduced power.

Discussion

Summary of current findings

This 11-year Chinese elderly cohort study provides the first epidemiologic evidence that dietary acrylamide exposure could potentially increase overall cancer mortality. The association was also present in current non-smokers.

Similar increased risks were observed in cancer mortality from digestive tract and respiratory system.

The average acrylamide intakes were 15.9 ± 8.5 $\mu\text{g/day}$ (0.26 $\mu\text{g/kg bw/day}$) for men and 13.2 ± 7.6 $\mu\text{g/day}$ (0.24 $\mu\text{g/kg bw/day}$) for women. The intake levels were similar to those reported in a previous survey in Hong Kong adults (Wong et al. 2014) (0.21 $\mu\text{g/kg bw/day}$) and in the Chinese general population (0.286 $\mu\text{g/kg bw/day}$) (Zhou et al. 2013); but were in the lower range of the WHO estimate of 0.3 – 0.8 $\mu\text{g/kg bw/day}$ for developed countries (Wong et al. 2014). This could be due to the lower intake of fried and baked foods among Chinese elderly compared with that of the Western populations (WHO/FAO 2002). Our results indicated the majority of the participants (82%) never consumed acrylamide-rich snack foods.

The major contributors of dietary acrylamide in our data were fried vegetables, baked cereals and their products. The findings were similar to that reported by the 1st Total Diet Study in Hong Kong adults (Wong et al. 2014). Although fried potato or snack foods such as potato chips and biscuits contain the highest acrylamide level, they had only a minor contribution to the overall exposure due to the low

Table 2 Characteristics of participants across quartiles of daily dietary acrylamide exposure ($\mu\text{g/day}$)

Variables	Q1	Q2	Q3	Q4	<i>P</i>
No.	1000	1000	1000	1000	
Acrylamide exposure range ($\mu\text{g/day}$)	0	9.94	12.94	17.09	
Average acrylamide intake ($\mu\text{g/day}$)	7.89 ± 1.61	11.47 ± 0.86	14.81 ± 1.18	24.20 ± 10.89	<0.001
Average acrylamide intake ($\mu\text{g/kg/day}$)	0.143 ± 0.037	0.202 ± 0.037	0.259 ± 0.049	0.410 ± 0.208	<0.001
Male/female (no.)	364/636	438/562	537/463	661/339	
Age (years)	73.3 ± 5.5	72.5 ± 5.2	72.3 ± 5.2	71.8 ± 4.8	<0.001
Education above university, <i>n</i> (%)	56 (5.6)	90 (9.0)	109 (10.9)	135 (13.5)	<0.001
Married or cohabitation, <i>n</i> (%)	605 (60.5)	673 (67.3)	739 (73.9)	812 (81.2)	<0.001
Body weight (kg)	56.4 ± 9.5	58.3 ± 9.4	58.7 ± 9.6	60.8 ± 10.2	<0.001
BMI (kg/m^2)	23.6 ± 3.4	23.8 ± 3.29	23.6 ± 3.2	23.9 ± 3.2	0.113
Smoking status, <i>n</i> (%)					<0.001
Current smoking	65 (6.5)	60 (6.0)	65 (6.5)	85 (8.5)	
Ever smoking	254 (25.4)	269 (26.9)	320 (32.0)	348 (34.8)	
No smoking	681 (68.1)	671 (67.1)	615 (61.5)	567 (56.7)	
Smoking dosage (packs/year)	9.4 ± 21.3	8.9 ± 20.0	11.4 ± 22.29	14.2 ± 27.1	<0.001
Physical activity (total PASE scores)	80.9 ± 36.3	90.1 ± 41.6	93.7 ± 45.2	101.2 ± 46.5	<0.001
Medical history at baseline, <i>n</i> (%)					
Any cancer	42 (4.2)	50 (5.0)	41 (4.1)	44 (4.4)	0.764
Bladder cancer	2 (0.2)	5 (0.5)	5 (0.5)	4 (0.4)	0.680
Diabetes	161 (16.1)	171 (17.1)	121 (12.1)	126 (12.6)	0.002
Stoke	53 (5.3)	35 (3.5)	44 (4.4)	43 (4.3)	0.274
CVDs	103 (10.3)	97 (9.7)	97 (9.7)	96 (9.6)	0.951
AHA-DLR score	42.1 ± 9.7	44.4 ± 10.0	44.8 ± 10.4	45.7 ± 9.7	<0.001
Dietary variables					
Total energy (kcal/day)	1395.9 ± 399.4	1677.1 ± 419.9	1945.3 ± 449.6	2349.1 ± 597.4	<0.001
Carbohydrate (g/day)	198.1 ± 60.1	237.6 ± 64.9	271.2 ± 73.8	320.3 ± 87.3	<0.001
Protein (g/1000 kcal)	37.4 ± 10.1	40.2 ± 8.9	41.8 ± 8.6	44.6 ± 8.7	<0.001
Fat (g/1000 kcal)	31.7 ± 8.1	31.1 ± 7.0	31.6 ± 6.9	31.5 ± 6.4	0.374
Fiber (g/day)	6.0 ± 3.4	8.0 ± 3.4	9.8 ± 4.3	13.1 ± 5.3	<0.001
Fruits (g/1000 kcal)	136.5 ± 95.2	144.7 ± 94.3	143.6 ± 91.3	149.1 ± 89.7	0.028
Vegetables (g/1000 kcal)	112.0 ± 55.1	144.4 ± 62.2	163.9 ± 73.6	211.3 ± 129.4	<0.001
Coffee (ml/day)	16.4 ± 58.0	20.0 ± 65.1	24.2 ± 66.7	34.7 ± 84.6	<0.001
Alcohol drinking (g/day)	7.1 ± 42.9	8.0 ± 44.1	12.3 ± 47.9	29.0 ± 146.7	<0.001
Tea (ml/week)	2986 ± 3267	3346 ± 3264	3801 ± 3928	4254 ± 4032	<0.001
French fries/chips (% Kcal)	0.07 ± 0.30	0.13 ± 0.95	0.23 ± 0.97	0.38 ± 1.21	<0.001
Fast food (% Kcal)	0.47 ± 1.30	0.54 ± 1.18	0.82 ± 2.51	0.80 ± 1.57	<0.001
Red/processed meat (% Kcal)	6.32 ± 5.13	6.94 ± 5.38	7.18 ± 4.77	7.52 ± 4.97	<0.001
SBP (mmHg)	144.1 ± 19.4	142.8 ± 18.2	142.1 ± 19.2	142.1 ± 19.7	0.076
DBP (mmHg)	77.9 ± 9.4	77.0 ± 9.2	78.3 ± 8.8	78.3 ± 9.3	0.007
Serum sex hormones among men (<i>n</i> = 1488)					
<i>n</i>	274	322	395	497	
SHBG (nmol/L)	43.5 ± 14.8	42.9 ± 17.8	42.0 ± 16.2	44.1 ± 17.5	0.822
Estradiol (pg/ml)	24.0 ± 8.4	24.5 ± 10.0	24.3 ± 10.2	26.1 ± 21.2	0.201
Bioavailable Estradiol (pmol/L)	77.4 ± 18.8	78.0 ± 21.9	77.0 ± 21.9	78.0 ± 22.0	0.981
Androstenedione (ng/ml)	0.73 ± 0.26	0.75 ± 0.22	0.73 ± 0.24	0.73 ± 0.22	0.482
Free androgen index	38.1 ± 12.0	39.4 ± 12.3	39.6 ± 11.2	40.3 ± 11.5	0.121
Dehydroepiandrosterone (ng/ml)	1.78 ± 1.05	1.95 ± 0.92	1.84 ± 0.99	1.97 ± 1.03	0.057

Table 2 (continued)

Variables	Q1	Q2	Q3	Q4	P
5-Androstene-3b,17b-diol (ng/ml)	0.67 ± 0.37	0.67 ± 0.35	0.68 ± 0.36	0.69 ± 0.34	0.826
Dehydroepiandrosterone sulfate (mg/ml)	0.85 ± 0.53	1.02 ± 0.59	0.92 ± 0.54	1.00 ± 0.53	<0.001

Data were presented as mean ± standard deviation for continuous variables or number (%) for categorical variables

ANOVA was used for continuous variables and Chi-square test was used for categorical variables

All hormone assays were performed by gas chromatography–mass spectrometry (GC/MS)

BMI body mass index, *SHBG* sex hormone-binding globulin, Free androgen index: calculated using (TT/SHBG × 100), *PASE* physical activity scale for the elderly, *AHA-DLR scores* adherence index of AHA diet and lifestyle recommendations, *SBP* systolic blood pressure, *DBP* diastolic blood pressure

Table 3 Hazard ratios (HRs and 95% CIs) of the association between dietary acrylamide exposure and cancer mortality from overall, digestive system and respiratory system and sensitivity analysis among current non-smokers by Cox regression model among Hong Kong elderly men and women

Acrylamide exposure	Q1	Q2	Q3	Q4	P for trend
Median (min–max) (µg/day)	8.16 (0–9.94)	11.48(9.94–12.94)	14.64 (12.94–17.09)	21.36 (17.09)	
Number	958	950	959	956	
Overall cancer mortality					
Cases/person-years	71/9488	73/9688	86/9969	100/10,116	
Crude HR (95% CI)	1.0 (reference)	0.9 (0.7–1.4)	1.1 (0.8–1.5)	1.3 (0.9–1.7)	0.077
Full adjusted HR (95% CI)	1.0 (reference)	1.2 (0.8–1.6)	1.4 (1.0–2.0)	1.9 (1.3–2.8)	0.001
Cancer mortality from digestive tract					
Cases/person-years	25/9488	31/9698	34/9969	41/10,116	
Crude HR (95% CI)	1.0 (reference)	1.2 (0.7–2.0)	1.3 (0.8–2.1)	1.5 (0.9, 2.4)	0.121
Full adjusted HR (95% CI)	1.0 (reference)	1.3 (0.8–2.2)	1.4 (0.8–2.5)	1.9 (1.0, 3.6)	0.052
Cancer mortality from respiratory system					
Cases/person-years	24/9488	22/9698	26/9969	34/10,116	
Crude HR (95% CI)	1.0 (reference)	0.9 (0.5–1.6)	1.0 (0.6–1.7)	1.3 (0.8–2.2)	0.289
Full adjusted HR (95% CI)	1.0 (reference)	1.2 (0.7–2.2)	1.3 (0.7–2.5)	2.0 (1.0–4.0)	0.056
Overall cancer mortality in non-current smokers					
Number	892	890	893	874	
Cases/person-years	62/8894	65/9173	70/9309	85/9271	
Crude HR (95% CI)	1.0 (reference)	1.0 (0.7–1.4)	1.1 (0.7–1.5)	1.3 (0.9–1.8)	0.130
Full adjusted HR (95% CI)	1.0 (reference)	1.2 (0.8–1.7)	1.4 (0.9–2.0)	2.0 (1.3–3.1)	0.002

Participants with baseline cancer history were excluded and 3823 were included for the analysis. Cox regression was used for the analysis among overall participants and participants who were non-current smokers. The adjusted variables (by entering method) in Cox regression model included: sex, age, education, total energy (kcal), BMI (kg/m²), smoking (no, occasional and current), total AHA-DRL score, tea drinking (ml/week), coffee (ml/day), physical activity (PASE total score), medical history of diabetes, dietary carbohydrate (g % kcal), dietary red or processed meats intake (% kcal) and dietary fiber intake (g/day)

consumption in the study participants. Based on our findings, mitigation procedures to minimize the formation of acrylamide in vegetables and cereals by reducing the cooking temperature and time, as well as sugars in foods could probably help to decrease the acrylamide exposure and cancer mortality in the Chinese elderly population.

Studies in rodents have shown positive dose–response relations between acrylamide exposure and cancer in multiple organs (Shipp et al. 2006). However, observational studies on dietary acrylamide intake and its relation with

cancer risk have reported inconsistent findings. Some indicated positive association between acrylamide exposure and increased risk of endometrial or ovarian cancer (Hogervorst et al. 2007; Wilson et al. 2010), renal cancer (Hogervorst et al. 2008a, b; Pelucchi et al. 2015), cutaneous malignant melanoma in men (Lipunova 2016), estrogen receptor positive breast cancer (Olesen et al. 2008), lymphatic malignancies (Bongers et al. 2012), colorectal cancer with specific mutations in KRAS and APC (Hogervorst et al. 2014) and lung cancer in smoking men (Hirvonen et al. 2010); but no

Table 4 Hazard ratios (HRs and 95% CIs) of the associations between acrylamide exposure and overall cancer mortality: subgroup analyses by sex (male and female), obesity (BMI < and ≥ 24) and total AHA-DRL score (< and > median) among Hong Kong elderly men and women

Acrylamide exposure	Q1	Q2	Q3	Q4	P for trend
Median (min–max) (μg/day)	8.16 (0–9.94)	11.48 (9.94–12.94)	14.64 (12.94–17.09)	21.36 (17.09)	
Sex (P for interaction = 0.259)					
Men					
N/cases/person-years	347/36/3335	414/43/4104	522/62/5383	630/72/6676	
Full adjusted HR (95% CI)	1.0 (reference)	1.3 (0.8, 2.0)	1.5 (1.0, 2.4)	1.9 (1.1, 3.1)	0.011
Women					
N/cases/person-years	611/35/6153	536/30/5593	437/24/4587	326/28/3440	
Full adjusted HR (95% CI)	1.0 (reference)	1.0 (0.6, 1.7)	1.1 (0.6, 2.0)	2.0 (1.0, 3.9)	0.059
BMI (P for interaction = 0.631)					
BMI < 24					
N/cases/person-years	543/36/5350	510/42/5149	553/49/5712	517/52/5494	
Full adjusted HR (95% CI)	1.0 (reference)	1.5 (1.0, 2.4)	1.6 (1.0, 2.6)	2.1 (1.2, 3.6)	0.011
BMI ≥ 24					
N/cases/person-years	415/35/4138	440/31/4549	406/37/4257	438/48/4612	
Full adjusted HR (95% CI)	1.0 (reference)	0.9 (0.5, 1.5)	1.3 (0.8, 2.1)	1.7 (0.9, 3.1)	0.049
AHA-DLR (P for interaction = 0.852)					
AHA < median					
N/cases/person-years	589/51/5750	481/40/4829	468/56/4800	446/61/4664	
Full adjusted HR (95% CI)	1.0 (reference)	1.1 (0.7, 1.6)	1.6 (1.0, 2.4)	2.0 (1.3, 3.3)	0.002
AHA ≥ median					
N/cases/person-years	364/20/3688	465/33/4825	490/30/5158	508/39/5439	
Full adjusted HR (95% CI)	1.0 (reference)	1.3 (0.8, 2.4)	1.2 (0.7, 2.3)	1.8 (0.9, 3.5)	0.107

Participants with baseline cancer history were excluded and 3823 were included for the analysis. The adjusted variables (by entering method) in Cox regression model included: sex, age, education, total energy (kcal), BMI (kg/m²), smoking (no, occasional and current), total AHA-DRL score, tea drinking (ml/week), coffee (ml/day), physical activity (PASE score), medical history of diabetes, red or processed meats (%kcal) and dietary fiber (g/day). For women, we further adjusted for years since menopause (years), age of first menstruation (years), years of contraceptives injection or drugs (years), years of estrogen use (years)

AHA-DLR scores adherence index of AHA diet and lifestyle recommendations

associations were observed for bladder and prostate cancer (Hogervorst et al. 2008a, b, Larsson et al. 2009), colorectal cancer (Mucci et al. 2006; Hogervorst et al. 2008a, b), lung cancer in men (Hogervorst et al. 2009), gastric, pancreatic and oesophageal cancers (Hogervorst et al. 2008a, b). A recent systematic review and meta-analysis (Pelucchi et al. 2015) of epidemiological studies indicated a borderline association with dietary acrylamide emerged for endometrial (RR = 1.23; 95% CI 1.00–1.51) and ovarian (RR = 1.39; 95% CI 0.97–2.00) cancers in never smokers, and a modest association for kidney cancer.

Studies that examined the relationship between dietary acrylamide and cancer mortality or prognosis were limited. Only one longitudinal study (Olsen et al. 2012) investigated the relationship of dietary acrylamide exposure and breast cancer mortality. The cohort of 420 postmenopausal Danish women of breast cancer indicated that pre-diagnostic acrylamide exposure reduced survival after breast cancer diagnosis (Olsen et al. 2012). Among non-smokers, higher concentrations of GA-Hb were associated with an increased

risk of breast cancer specific mortality [HR (95% CI) 1.63 (1.06–2.51)] (Olsen et al. 2012). Our results revealed that dietary acrylamide exposure was associated with increased overall cancer mortality. More research is needed to explore the associations of acrylamide exposure with specific cancer mortality or prognosis.

Mechanisms

The biological mechanism by which acrylamide causes cancer development in humans is yet unclear. Both genotoxic and non-genotoxic pathways have been suggested (Besaratina and Pfeifer 2007). Acrylamide itself and its epoxide metabolite glycidamide, which is generated by cytochrome P4502E1 (CYP2E1), are clastogenic, and glycidamide forms DNA adducts. Acrylamide may also cause cancer through non-genotoxic mechanisms. Oxidative stress, following depletion of glutathione by acrylamide, is one of the proposed mechanisms. Acrylamide reacts with glutathione and may thus alter the redox status of cells

and gene transcription and expression, facilitating cancer development (Catalgol et al. 2009), or it may interfere with DNA repair or hormonal balances (Besaratnia and Pfeifer 2007). In some test systems, aneuploidy was observed upon acrylamide incubation, which might be caused by binding of acrylamide to proteins involved in cell division, such as mitotic/meiotic spindle kinesins (Sickles et al. 2007).

Acrylamide may also be carcinogenic through hormonal pathways (Hogervorst et al. 2007). Our results also suggested that additional adjustment of free estradiol level in multivariable regression model reduced the HRs of dietary acrylamide and cancer mortality to around 1. Acrylamide has been shown to alter steroid hormone levels in premenopausal (Nagata et al. 2015) and postmenopausal women (Hogervorst et al. 2013). There could be interactions between acrylamide intake and genes involved in the generation of sex hormones (Hogervorst et al. 2016; Hogervorst et al. 2017). The influence on circulating estrogen levels is the key mechanism behind several of the factors known to be associated with breast cancer incidence and/or prognosis (Kendall et al. 2007; Folkert and Dowsett 2010).

Strengths

The strengths of this study include the use of a validated FFQ for acrylamide assessment, the prospective nature precluding selection and recall bias, the long duration of follow-up, and the completeness of case ascertainment through linkage to the registries of the Health Authority.

We assessed acrylamide intake based on an analytical acrylamide database with acrylamide values derived from an extensive and elaborate sampling scheme and chemical analysis of all relevant Hong Kong foods. A previous study suggested that using mean acrylamide levels in foods to estimate the total acrylamide intake could be a valid approach (Zhou et al. 2013). For estimating the long-term exposure to the usual acrylamide intake in our study, the mean acrylamide levels for foods are expected to be even more suitable. Finally, protopathic bias was probably not present in our prospective cohort study, as exclusion of death cases in the first 2 years of follow-up did not alter the conclusions.

Limitations

This study has several limitations. First, as in most of epidemiological studies, we used a validated FFQ to estimate dietary acrylamide exposure. Although the use of FFQs has limitations of possible misclassification, they are the only feasible way in large epidemiological studies to assess the intake of the relevant acrylamide-containing foods over a long time period (Wilson et al. 2009). In addition, the non-differential misclassification could only bias the risk estimates toward null. Although acrylamide adducts to

hemoglobin are recognized as the internal dose markers of ‘exposure’ to acrylamide (Dybing et al. 2005), they represent the exposure during the preceding 3–4 months only. The biomarker is not specific with regard to the source of acrylamide. The costs of using biomarkers also limit the size of the population that can be used. In multivariable regression models, we further adjusted for dietary carbohydrate and fibers, the nutrients that are correlated to acrylamide, which suggested that the significant associations of acrylamide and cancer mortality could be due to the acrylamide itself, but not dietary carbohydrate and fiber levels. In addition, dietary intakes by FFQ were only assessed at baseline; we did not conduct the same dietary survey during follow-up. The food intakes that were investigated in 2001–2003 may not be completely representative of the foods that were on the market during follow-up. It has been shown that after 2005, acrylamide levels in some products have decreased (Stadler 2005). However, the reduction of acrylamide mostly occurred in fried potatoes, which had a minor contribution to overall acrylamide exposure in our study population since they had low intake of fried or baked food or snacks.

Second, although both industrial contact and tobacco smoking are important sources of environmental acrylamide exposure (Wirfalt et al. 2008), exposure from diet and drinking water was the major route of acrylamide in our elderly participants since they were non-occupationally exposed and had low prevalence of current smoking (6.8%).

Third, we have not collected the data on new cancer cases during follow-up but only data on cancer mortality. In addition, we did not analyze the association of acrylamide exposure with each kind of cancer mortality due to insufficient individual cancer deaths. However, animal studies have demonstrated that acrylamide-induced cancers affected various tissues. This is because the molecule of acrylamide is small and hydrophilic, it passively diffuses throughout the body and all tissues are theoretical targets for acrylamide carcinogenesis (Friedman 2003). Our subgroup analyses on mortality in either digestive or respiratory system reported similar findings on the associations of acrylamide exposure and cancer deaths. Another limitation is that we did not collect the treatment information after cancer occurrence. However, the treatment is unlikely to be correlated with acrylamide intake, and thus the association would be reduced towards the null. Future studies on acrylamide and cancer mortality should take treatment into account.

Finally, the study was conducted by non-random sampling in a single center in Hong Kong, so the findings may not be generalizable to ethnic Chinese elsewhere. Additionally, potential residual confounders from unmeasured factors such as past occupational exposure to carcinogens and concomitant carcinogens from foods are possible. However, the increased strength of associations after multivariable adjustment indicates that residual confounding by the covariables

is probably not the explanation for the observed acrylamide-associated associations.

Conclusions

Dietary exposure to acrylamide in amounts typically ingested by Chinese elderly was found to be significantly associated with increased overall cancer mortality, and mortality from cancers of the digestive tract and respiratory system. Circulating free estradiol may mediate this association in men. Further prospective studies specifically conducted among new cases of certain cancer are necessary to confirm these findings.

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Author contributions ZML conceptualized the study, analyzed the data, interpreted the results, and drafted the manuscript. Suyang Wu helped in the calculation of dietary acrylamide exposure. All the coauthors critically commented on and revised the manuscript.

Compliance with ethical standards

Conflict of interest None of the authors have competing interests to report.

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References

Besaratinia A, Pfeifer GP (2007) A review of mechanisms of acrylamide carcinogenicity. *Carcinogenesis* 28(3):519–528

Bongers ML, Hogervorst JG, Schouten LJ, Goldbohm RA, Schouten HC, van den Brandt PA (2012) Dietary acrylamide intake and the risk of lymphatic malignancies: the Netherlands Cohort Study on diet and cancer. *PLoS One* 7(6):e38016

Bull RJ, Robinson M, Stober JA (1984) Carcinogenic activity of acrylamide in the skin and lung of Swiss-ICR mice. *Cancer Lett* 24(2):209–212

Catalgol B, Ozhan G, Alpertunga B (2009) Acrylamide-induced oxidative stress in human erythrocytes. *Hum Exp Toxicol* 28(10):611–617

Centre for Food Safety, Food and Environmental Hygiene Department (2011) The first Hong Kong Total Diet Study. *Methodology* 1–34

Dybing E, Farmer PB, Andersen M, Fennell TR, Lalljie SP, Müller DJ, Olin S, Petersen BJ, Schlatter J, Scholz G, Scimeca JA, Slimani N, Törnqvist M, Tuijelaars S, Verger P (2005) Human exposure and internal dose assessments of acrylamide in food. *Food Chem Toxicol* 43(3):365–410

Folkert EJ, Dowsett M (2010) Influence of sex hormones on cancer progression. *J Clin Oncol* 28(26):4038–4044

Friedman M (2003) Chemistry, biochemistry, and safety of acrylamide. A review. *J Agric Food Chem* 51(16):4504–4526

Hirvonen T, Kontto J, Jestoi M, Valsta L, Peltonen K, Pietinen P, Virtanen SM, Sinkko H, Kronberg-Kippila C, Albanes D, Virtamo J (2010) Dietary acrylamide intake and the risk of cancer among Finnish male smokers. *Cancer Causes Control* 21(12):2223–2229

Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2007) A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev* 16(11):2304–2313

Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2008a) Dietary acrylamide intake and the risk of renal cell, bladder, and prostate cancer. *Am J Clin Nutr* 87(5):1428–1438

Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2008b) Dietary acrylamide intake is not associated with gastrointestinal cancer risk. *J Nutr* 138(11):2229–2236

Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2009) Lung cancer risk in relation to dietary acrylamide intake. *J Natl Cancer Inst* 101(9):651–662

Hogervorst JG, Fortner RT, Mucci LA, Tworoger SS, Eliassen AH, Hankinson SE, Wilson KM (2013) Associations between dietary acrylamide intake and plasma sex hormone levels. *Cancer Epidemiol Biomarkers Prev* 22(11):2024–2036

Hogervorst JG, de Bruijn-Geraets D, Schouten LJ, van Engeland M, de Kok TM, Goldbohm RA, van den Brandt PA, Weijenberg MP (2014) Dietary acrylamide intake and the risk of colorectal cancer with specific mutations in KRAS and APC. *Carcinogenesis* 35(5):1032–1038

Hogervorst JG, van den Brandt PA, Godschalk RW, van Schooten FJ, Schouten LJ (2016) The influence of single nucleotide polymorphisms on the association between dietary acrylamide intake and endometrial cancer risk. *Sci Rep* 6:34902

Hogervorst JG, van den Brandt PA, Godschalk RW, van Schooten FJ, Schouten LJ (2017) Interactions between dietary acrylamide intake and genes for ovarian cancer risk. *Eur J Epidemiol* 32(5):431–441

JECFA (2005) Summary and conclusions of the sixty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 1–47

Johnson KA, Gorzinski SJ, Bodner KM, Campbell RA, Wolf CH, Friedman MA, Mast RW (1986) Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol Appl Pharmacol* 85(2):154–168

Kendall A, Folkert EJ, Dowsett M (2007) Influences on circulating oestrogens in postmenopausal women: relationship with breast cancer. *J Steroid Biochem Mol Biol* 103(2):99–109

Labrie F, Cusan L, Gomez JL, Martel C, Berube R, Belanger P, Belanger A, Vandenput L, Mellstrom D, Ohlsson C (2009) Comparable amounts of sex steroids are made outside the gonads in men and women: strong lesson for hormone therapy of prostate and breast cancer. *J Steroid Biochem Mol Biol* 113(1–2):52–56

Larsson SC, Akesson A, Wolk A (2009) Dietary acrylamide intake and prostate cancer risk in a prospective cohort of Swedish men. *Cancer Epidemiol Biomarkers Prev* 18(6):1939–1941

Lau EM, Leung PC, Kwok T, Woo J, Lynn H, Orwoll E, Cummings S, Cauley J (2006) The determinants of bone mineral density in Chinese men—results from Mr. Os (Hong Kong), the first cohort study on osteoporosis in Asian men. *Osteoporos Int* 17(2):297–303

Lipunova N, Schouten LJ, van den Brandt PA, Hogervorst JG (2016) A prospective cohort study on dietary acrylamide intake and the risk for cutaneous malignant melanoma. *Eur J Cancer Prev* doi:10.1097/CEJ.0000000000000268

Mottram DS, Wedzicha BL, Dodson AT (2002) Acrylamide is formed in the Maillard reaction. *Nature* 419(6906):448–449

- Mucci LA, Adami HO, Wolk A (2006) Prospective study of dietary acrylamide and risk of colorectal cancer among women. *Int J Cancer* 118(1):169–173
- Nagata C, Konishi K, Tamura T, Wada K, Tsuji M, Hayashi M, Takeda N, Yasuda K (2015) Associations of acrylamide intake with circulating levels of sex hormones and prolactin in premenopausal Japanese women. *Cancer Epidemiol Biomarkers Prev* 24(1):249–254
- Olesen PT, Olsen A, Frandsen H, Frederiksen K, Overvad K, Tjønneland A (2008) Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study. *Int J Cancer* 122(9):2094–2100
- Olsen A, Christensen J, Outzen M, Olesen PT, Frandsen H, Overvad K, Halkjaer J (2012) Pre-diagnostic acrylamide exposure and survival after breast cancer among postmenopausal Danish women. *Toxicology* 296(1–3):67–72
- Pelucchi C, La Vecchia C, Bosetti C, Boyle P, Boffetta P (2011) Exposure to acrylamide and human cancer—a review and meta-analysis of epidemiologic studies. *Ann Oncol* 22(7):1487–1499
- Pelucchi C, Bosetti C, Galeone C, La Vecchia C (2015) Dietary acrylamide and cancer risk: an updated meta-analysis. *Int J Cancer* 136(12):2912–2922
- Shipp A, Lawrence G, Gentry R, McDonald T, Bartow H, Bounds J, Macdonald N, Clewell H, Allen B, Van Landingham C (2006) Acrylamide: review of toxicity data and dose-response analyses for cancer and noncancer effects. *Crit Rev Toxicol* 36(6–7):481–608
- Sickles DW, Sperry AO, Testino A, Friedman M (2007) Acrylamide effects on kinesin-related proteins of the mitotic/meiotic spindle. *Toxicol Appl Pharmacol* 222(1):111–121
- Södergard R, Bäckström T, Shanbhag V, Carstensen H (1982) Calculation of free and bound fractions of testosterone and estradiol-17 β to human plasma proteins at body temperature. *J Steroid Biochem* 16(6):801–810
- Stadler RH (2005) Acrylamide formation in different foods and potential strategies for reduction. *Adv Exp Med Biol* 561:157–169
- Stewart BW, Wild CP (2014) World Cancer Report 2014
- Wilson KM, Vesper HW, Tocco P, Sampson L, Rosén J, Hellenäs KE, Törnqvist M, Willett WC (2009) Validation of a food frequency questionnaire measurement of dietary acrylamide intake using hemoglobin adducts of acrylamide and glycidamide. *Cancer Causes Control* 20(3):269–278
- Washburn RA, Smith KW, Jette AM, Janney CA (1993) The Physical Activity Scale for the Elderly (PASE): development and evaluation. *J Clin Epidemiol* 46(2):153–162
- WHO/FAO (2002) Health implications of acrylamide in food. Report of a Joint FAO/WHO Consultation. WHO. <http://apps.who.int/iris/bitstream/10665/42563/1/9241562188.pdf>. Accessed 19 July 2017
- Wilson KM, Mucci LA, Rosner BA, Willett WC (2010) A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancers. *Cancer Epidemiol Biomarkers Prev* 19(10):2503–2515
- Wirfalt E, Paulsson B, Tornqvist M, Axmon A, Hagmar L (2008) Associations between estimated acrylamide intakes, and hemoglobin AA adducts in a sample from the Malmo Diet and Cancer cohort. *Eur J Clin Nutr* 62(3):314–323
- Wong WW, Chung SW, Lam CH, Ho YY, Xiao Y (2014) Dietary exposure of Hong Kong adults to acrylamide: results of the first Hong Kong Total Diet Study. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 31(5):799–805
- Woo J, Leung SSF, Ho SC, Lam TH, Janus ED (1997) A food frequency questionnaire for use in the Chinese population in Hong Kong: description and examination of validity. *Nutr Res* 17(11):1633–1641
- Zhou PP, Zhao YF, Liu HL, Ma YJ, Li XW, Yang X, Wu YN (2013) Dietary exposure of the Chinese population to acrylamide. *Biomed Environ Sci* 26(6):421–429

**Dietary Acrylamide Intake and Risk of Esophageal, Gastric, and Colorectal Cancer:
The Japan Public Health Center-based Prospective Study**

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Abstract

Background: Acrylamide has been classified as a probable human carcinogen based chiefly on laboratory evidence. However, the influence of dietary acrylamide intake on risk of esophageal, gastric, and colorectal cancer has not been extensively studied. We aimed to evaluate the association between dietary acrylamide intake and esophageal, gastric, and colorectal cancer using data from the Japan Public Health Center-based Prospective Study.

Methods: Our study included 87,628 participants who completed a food-frequency questionnaire at enrollment in 1990 and 1993. We used Cox proportional hazards regression models to estimate hazards ratios and 95% confidence intervals (CIs) after adjusting for confounding factors.

Results: After 15.5, 15.3, and 15.3 mean years of follow-up for esophageal, gastric, and colorectal cancer, we identified and analyzed 391 esophageal, 2,218 gastric, and 2,470 colorectal cancer cases, respectively. Compared with the lowest quintile of acrylamide intake, the multivariate hazard ratio for the highest quintile was 0.86 (95% CI 0.53-1.39, P for trend=0.814), 0.84 (95% CI 0.69-1.01, P for trend=0.301), and 0.93 (95% CI 0.79-1.08, P for trend=0.165) for esophageal, gastric, and colorectal cancer, respectively, in the multivariable-adjusted model. Furthermore, no significant associations were observed when the participants were stratified by cancer subsites.

Conclusions: In conclusion, this study demonstrated that dietary acrylamide intake was not associated with increased risk of esophageal, gastric, or colorectal cancer among the Japanese population.

Impact: It is the first time to assess the effect of dietary acrylamide intake on risk of digestive system cancer in Asian populations.

1

2

3 **Keywords:** dietary acrylamide, esophageal cancer, gastric cancer, colorectal cancer,
4 epidemiology

5

1 Introduction

2 Acrylamide, an important industrial monomer, is widely used in the manufacture of
3 water-soluble polymers used for water treating, paper, mining, and sugar processing (1-
4 5). In 1994, acrylamide was classified as a probable human carcinogen (Group 2A) by
5 the International Agency for Research on Cancer (IARC) based on laboratory studies (6).
6 For a long time, specific occupations and smoking were thought to be the main sources
7 of acrylamide exposure (Food Safety Commission of Japan. Evaluation document of
8 dietary acrylamide produced by heating. Tokyo: Food Safety Commission of Japan **2016**;
9 https://www.fsc.go.jp/osirase/acrylamide1.data/acrylamide_hyokasyo1.pdf). In 2002,
10 Swedish investigators reported that acrylamide is formed in commonly consumed starchy
11 foods cooked at high temperature ($>120^{\circ}\text{C}$), suggesting that the meals are another main
12 source of acrylamide (7). The carcinogenic effect of dietary acrylamide is considered to
13 occur through a genotoxic pathway (8). Acrylamide has a small hydrophilic molecule and
14 can reach every organ and tissue in the body. Therefore, theoretically, all tissues can be
15 targets for carcinogenesis due to acrylamide. There are two metabolic pathways for
16 acrylamide, a direct pathway of glutathione conjugation of acrylamide by GST, and a
17 secondary pathway of glycidamide by cytochrome P450 and conjugation by GST. Both
18 acrylamide and glycidamide are capable of combining with DNA and lead to genotoxicity
19 (9).

20 The association between acrylamide exposure in occupational settings and risk of
21 cancers has been extensively studied (1-5); however, the results do not support the
22 conclusion that acrylamide is an occupational carcinogen. Meanwhile, epidemiologic
23 studies conducted in Western countries have reported that dietary acrylamide is not
24 associated with increased risk of most cancers. However, borderline associations with

1 dietary acrylamide were observed for kidney cancer, and for endometrial and ovarian
2 cancers in never smokers only (10).

3 Thus far, there are four (11-14), two (12,15), and six studies (11,12,15-18) examining
4 the relationship between dietary acrylamide exposure and esophageal, gastric, and
5 colorectal cancer, respectively. However, these epidemiological studies were all
6 conducted in Western countries. To our knowledge, no study has assessed the effect of
7 dietary acrylamide intake on the risk of esophageal, gastric, or colorectal cancer in Asian
8 populations. In addition, the main sources of dietary acrylamide are coffee and green tea,
9 followed by confectioneries, vegetables, and potatoes in Japan, while in Western countries,
10 they are potato-based foods, wheat-based products, and coffee (19). Therefore, it is
11 necessary to examine the influence of acrylamide intake on cancers in various countries
12 with different dietary sources of the chemical. This study aimed to investigate the
13 association between dietary acrylamide intake and risk of esophageal, gastric, and
14 colorectal cancer among the Japanese population.

Materials and Methods

Study cohorts and participants

This study was based on the Japan Public Health Center-based Prospective Study (JPHC Study), whose design was previously reported in detail (20). The JPHC Study began in 1990 (Cohort I) and 1993 (Cohort II), covering 11 public health center areas throughout Japan and including 140,420 residents (68,722 men and 71,698 women) aged 40-69 years. The JPHC Study aimed to investigate the association between lifestyle and lifestyle diseases, providing evidence for prevention and control of cancer and cardiovascular disease. A self-administered lifestyle questionnaire was delivered to all participants. Vital status, mortality, migration, and incidence of cancer and cardiovascular disease were recorded for every participant. A follow-up survey that included the lifestyle questionnaire was conducted 5 years after the baseline survey and 5 years after each succeeding survey. Particularly, a dietary survey using a self-administered food frequency questionnaire (FFQ) was conducted at baseline and at 5- and 10-year follow-ups. The FFQ of the 5-year follow-up survey obtained more-detailed dietary information than that of the baseline survey since it included more food items and portion size options. Therefore, we used the 5-year follow-up survey as the starting point of our study.

Residents who were aged 40 and 50 years were asked to enroll Medical Examination conducted by the local health center of age-biased areas in 1990 and 1993. Then, the residents who took part in Medical Examination were included in JPHC study. Therefore, we excluded participants in age-biased cohort areas where the participants were aged 40 and 50 years when they received the baseline questionnaire. We also excluded participants who did not meet the follow-up criteria and did not respond to the 5-year follow-up survey (Figure 1). A total of 94,816 participants completed the questionnaire.

Participants were also excluded from analysis if they had a history of esophageal, gastric, and colorectal cancer before the baseline survey (N=907) or during the time from the baseline survey to the 5-year follow-up survey (N=579), if they were lost to follow-up (N=37), and if they did not provide complete dietary data (those whose total energy intake could not be calculated or who reported extremely low or high energy values; N=5,665). Finally, a total of 87,628 subjects (40,732 men and 46,896 women) were eligible for analysis (Figure 1).

This study was approved by the Institutional Review Boards of the National Cancer Center Japan, Osaka University, and Azabu University.

Assessment of acrylamide intake

The FFQ was used to estimate nutrient and food intake among the subjects of the JPHC Study. Information regarding the usual consumption of 147 food items during the previous year was collected (21). Food intake was categorized into nine frequencies (never, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, once/day, 2–3 times/day, 4–6 times/day, and ≥ 7 times/day). Portion sizes were specified in three categories (less than half the standard portion size, standard portion size, and >1.5 times the standard portion size). The FFQ was previously validated by comparing intake with 28-day weighted dietary records (DRs) as reference in a subcohort of the JPHC Study (21-23). Daily nutrient intake was calculated based on the Fifth Revised and Enlarged Edition of the Standard Tables of Food Composition in Japan (5th FCT) (24).

Acrylamide intake was estimated using an acrylamide database (25) developed from measured values of acrylamide content in common Japanese foods reported elsewhere (26-30; Ministry of Agriculture, Forestry and Fisheries. Risk profile sheet relating to the

1 food safety; for acrylamide. Ministry of Agriculture, Forestry and Fisheries **2015**;
2 http://www.maff.go.jp/j/syouan/seisaku/risk_analysis/priority/pdf/150807_rp_aa.pdf;
3 National Institute for Environmental Studies, Japan. Study on statistical estimate of
4 acrylamide intake from foods. National Institute for Environmental Studies, Japan **2015**;
5 <http://www.fsc.go.jp/fsciis/technicalResearch/show/cho99920141408>; National Institute
6 of Health Sciences. Acrylamide analysis in food. **2016**;
7 <http://www.mhlw.go.jp/topics/2002/11/tp1101-1a.html>; Food Safety Commission of
8 Japan. Study on estimate of acrylamide intake from food; interim report. Food Safety
9 Commission of Japan **2016**;
10 <https://www.fsc.go.jp/fsciis/technicalResearch/show/cho99920151507>; Food Safety
11 Commission of Japan. Information clearing sheet for acrylamide. Food Safety
12 Commission of Japan **2016**;
13 [https://www.fsc.go.jp/fsciis/attachedFile/download?retrievalId=kai20111222sfc&fileId=](https://www.fsc.go.jp/fsciis/attachedFile/download?retrievalId=kai20111222sfc&fileId=520)
14 [520](https://www.fsc.go.jp/fsciis/attachedFile/download?retrievalId=kai20111222sfc&fileId=520)). Briefly, first, acrylamide-containing foods were identified from the foods listed in
15 the 5th FCT. Out of 1,878 food items in the 5th FCT, 282 food items were designated as
16 acrylamide-containing foods, 1,276 were non-acrylamide-containing foods, and 320 were
17 not classifiable. Further, because the acrylamide concentration of the same food differed
18 depending on the cooking method, 39 heated food items were added to the items.
19 Therefore, there were 321 food items (17% of total food items) considered as acrylamide-
20 containing foods for estimating DR-derived acrylamide intake (25). The development of
21 the acrylamide database was finished by linking the food list and measured values of
22 acrylamide content reported previously (26-30; Ministry of Agriculture, Forestry and
23 Fisheries. Risk profile sheet relating to the food safety; for acrylamide. Ministry of
24 Agriculture, Forestry and Fisheries **2015**;

http://www.maff.go.jp/j/syouan/seisaku/risk_analysis/priority/pdf/150807_rp_aa.pdf;
National Institute for Environmental Studies, Japan. Study on statistical estimate of
acrylamide intake from foods. National Institute for Environmental Studies, Japan **2015**;
<http://www.fsc.go.jp/fsciis/technicalResearch/show/cho99920141408>; National Institute
of Health Sciences. Acrylamide analysis in food. **2016**;
<http://www.mhlw.go.jp/topics/2002/11/tp1101-1a.html>; Food Safety Commission of
Japan. Study on estimate of acrylamide intake from food; interim report. Food Safety
Commission of Japan **2016**;
<https://www.fsc.go.jp/fsciis/technicalResearch/show/cho99920151507>; Food Safety
Commission of Japan. Information clearing sheet for acrylamide. Food Safety
Commission of Japan **2016**;
[https://www.fsc.go.jp/fsciis/attachedFile/download?retrievalId=kai20111222sfc&fileId=](https://www.fsc.go.jp/fsciis/attachedFile/download?retrievalId=kai20111222sfc&fileId=520)
[520](https://www.fsc.go.jp/fsciis/attachedFile/download?retrievalId=kai20111222sfc&fileId=520)).

In the food list of the FFQ, 28 (19%) out of 147 food items were also identified as
acrylamide-containing foods. The amount of raw food intake is usually used for
calculating most nutrient intakes in the FFQ; however, the amount of acrylamide present
differs between cooking methods. Therefore, FFQ-derived acrylamide intake was
calculated by considering cooking methods for following food items and using the
proportion of these heated food calculated from the DR: starchy vegetables (potato, sweet
potato), vegetables (onion, bean sprouts, sweet pepper, squash, cabbage, snap beans,
broccoli), rice (toast, boiled, or stir-fried), and fried batter (Food Safety Commission of
Japan. Study on estimate of acrylamide intake from food; interim report. Food Safety
Commission of Japan **2016**;
<https://www.fsc.go.jp/fsciis/technicalResearch/show/cho99920151507>). The

Spearman's correlation coefficients for energy-adjusted dietary acrylamide intake between DRs and FFQ ranged from 0.34 to 0.48 (25).

Follow-up and identification of cancer cases

The subjects were followed from the start of the 5-year follow-up survey until December 31, 2013. Residential status was confirmed annually through the residential registry. During the study period, 4,991 subjects (5.7%) moved out of the study area and 14,714 (16.8%) died.

Cases were identified from major local hospitals through data linkage with population-based cancer registries. Because in some study areas the completion of the cancer registry was low, members of the JPHC research group checked the clinical records of major local hospitals in these areas and compiled active patient identification for cancers, including date of diagnosis, diagnostic method, diagnostic name, International Classification of Disease for Oncology Codes (Third Edition), histologic type, and histologic codes, among other data. Death certificates were used as a supplementary source of information. The endpoints of this analysis were incidences of esophageal, gastric, and colorectal cancer defined as the ICD-O-3 (International Classification of Diseases for Oncology, Third Edition) codes C15, C16, and C18-C20, respectively. Until the end of the follow-up period, 391 esophageal, 2,218 gastric, and 2,470 colorectal cancer cases were newly identified in the study population. Among 391 esophageal cancer cases, there were 20 adenocarcinomas (EAC, M8140, 8211, 8260, 8560), 305 squamous cell carcinomas (ESCC, M8070), and 66 unclassified cases. Among the 2,218 gastric cancer cases, 138 cardia gastric cancer cases (CGC, ICD-O-3 C16) and 2080 non-cardia gastric cancer cases (NCGC, ICD-O-3 C16.1-C16.9) were identified. Of

the 2470 colorectal cancers, there were 1,721 colon cancer cases (ICD-O-3 C18) and 749 rectal cancer cases (ICD-O-3 C19, C20).

Statistical analysis

Person-years of follow-up for each subject were calculated from the start of the 5-year follow-up survey to the date of diagnosis of esophageal or gastric or colorectal cancer, date of death from any cause, date of relocation from the study area, or end of follow-up (December 31, 2013), whichever came first. The mean follow-up period was 15.5 years for esophageal cancer, 15.3 years for gastric cancer, and 15.3 years for colorectal cancer.

Our study used the residual method to adjust acrylamide intake by energy intake. Subjects were divided into quintiles (i.e., Q1, Q2, Q3, Q4, and Q5 groups) according to energy-adjusted intakes of acrylamide. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) to determine the association between quintiles of energy-adjusted dietary acrylamide intake and incidence of esophageal, gastric, or colorectal cancer, with Q1 as the reference group. Trends were assessed by assigning ordinal values for the quintiles of energy-adjusted acrylamide intake. Based on the 5-year follow-up survey, characteristics of dietary and non-dietary variables were compared between quintiles, Q1, Q2, Q3, Q4, and Q5 using the Kruskal-Wallis test or chi-squared test as appropriate.

The common (based on literature) potential confounders in multivariable-adjusted model were age (continuous), sex, area (10 public health center area), body mass index (<23.0, 23.0-24.9, 25.0-26.9, ≥ 27.0 kg/m², or missing), smoking status (never, former, current, or missing), pack years (<10.0, 10.0-19.9, 20.0-29.9, 30.0-39.9, ≥ 40.0 , or missing), physical activity (continuous), alcohol intake (<150 or ≥ 150 g/week). The

1 confounders that were energy-adjusted consumption of food and beverage (in grams per
2 day, continuous) were different in multivariable-adjusted model according to type of
3 cancer studied. Particularly, vegetables, fruits, and dairy were adjusted for esophageal
4 cancer; vegetables, fruits, and salted fish, for gastric cancer; and vegetables, fruits, meat,
5 and dairy, for colorectal cancer (see footnotes of Tables 2, 3, 4). In a sensitivity analysis,
6 we excluded 53 esophageal, 332 gastric, and 292 colorectal cancer cases that were
7 diagnosed in the first 3 years of follow-up. We also conducted an analysis that did not
8 include esophageal, gastric, or colorectal carcinoma in situ. In addition, considering
9 differences in risk factors between the subsites of cancers studied, we further performed
10 stratified analyses for ESCC and EAC, CGC and NCGC, and colon and rectal cancer.
11 Concerning esophageal cancer, obesity is a risk factor for EAC but not ESCC, while
12 alcohol use is a risk factor for ESCC but not EAC (31). On the other hand, obesity is also
13 a risk factor for CGC (32). Regarding colorectal cancer, physical activity is more strongly
14 associated with colon cancer rather than rectal cancer (33). Therefore, risk factors in the
15 stratified analysis of subsites were also adjusted accordingly (see footnotes of Tables 2,
16 3, 4).

17 Cigarette smoke is a major source of acrylamide, and smokers have on average 4
18 times higher levels of acrylamide-hemoglobin adducts (which mark internal acrylamide
19 dose) than nonsmokers (34). To elucidate the interaction effect, subgroup analyses were
20 conducted for never smoker, and former and current smokers. We also performed
21 stratified analysis for alcohol consumption (<150 or ≥ 150 g/week), coffee consumption
22 (never drinker or drinker), and green tea consumption (never drinker or drinker). All *P*
23 values were two-sided, with significance set at <0.05 . All statistical analyses were
24 performed with Stata version 13.1 (Stata Corp., College Station, TX, USA).

Results

Characteristics of the study population according to acrylamide intake are shown in Table

1. The mean (\pm standard deviation) daily intake of acrylamide was 6.8 ± 3.8 $\mu\text{g/day}$ overall, corresponding to 0.13 ± 0.16 $\mu\text{g/kg}$ body weight/day. Foods that mainly contributed to total acrylamide intake were coffee (28%), green tea (22%), biscuits (11%), potatoes (11%), and vegetables (11%). Compared with the lowest acrylamide consumption group (Q1), the highest consumption group (Q5) comprised younger subjects, a larger proportion of current smokers, and a higher number of pack years. Moreover, the food and beverage consumption of the Q5 group consisted of more coffee, green tea, vegetables, potatoes, fruits, and biscuits but less alcohol, meat, fish, dairy, soy food, and energy diet.

Table 2 shows the results of daily acrylamide intake and risk of esophageal cancer. No association between daily acrylamide intake and esophageal cancer was observed in the overall analysis ($P=0.814$). There were also no significant associations observed in the sensitivity analysis and the analysis excluding carcinoma in situ as well as in the stratified analysis.

Table 3 shows the associations between daily acrylamide intake and gastric cancer. Overall, acrylamide intake was not associated with total gastric cancer, cardia gastric cancer, or non-cardia gastric cancer. The sensitivity analysis and the analysis excluding carcinoma in situ also showed no significant associations.

Lastly, Table 4 shows the comparison between daily acrylamide intake and colorectal cancer. Daily acrylamide intake was significantly associated with decreased risk of colorectal cancer in the age- and area-adjusted model. Subjects in the highest acrylamide intake group (Q5) had approximately 11% lower risk of colorectal cancer than those in the lowest acrylamide intake group (Q1) (HR=0.89, 95% CI 0.78-1.01). However, after

1 additionally adjusting for factors in the multivariable-adjusted model, the significance of
2 the decreased association was attenuated and no significant association was observed.
3 The results did not change in the sensitivity analysis and when cases of colorectal
4 carcinoma in situ were excluded. Furthermore, colon or rectal cancer was also not
5 associated with acrylamide intake.

6 Supplementary tables S1-3 displayed the associations between daily dietary
7 acrylamide intake and esophageal, gastric and colorectal cancer with stratification
8 analyses by smoking status, alcohol consumption, coffee consumption, green tea
9 consumption, respectively (Supplementary Tables S1–3). In general, there were also no
10 significant associations observed in these analyses.

Discussion

Based on the large-scale prospective cohort of the JPHC Study, we found no association between dietary acrylamide intake and overall risk of esophageal, gastric, or colorectal cancer among the Japanese population.

Importantly, our results were almost consistent with those of previous studies. To date, two case-control studies (11,13) and two cohort studies (12,14) have evaluated dietary acrylamide intake and risk of esophageal cancer. In these four studies, there was no overall association between acrylamide intake and risk of esophageal cancer, and the summary risk ratio (RR) for high versus low level of acrylamide intake was 1.14 (95% CI 0.93-1.38, P for trend=0.41) (10). Two prospective cohort studies (14,15) also did not support an association between dietary acrylamide intake and risk of gastric cancer, and in these two studies, the summary RR for high versus low level of acrylamide intake was 1.03 (95% CI 0.94-1.10, P for trend=0.73) (10). In this study, dietary acrylamide intake was also not associated with gastric cancer risk in overall analysis. Meanwhile, six European studies, two case-control studies (11,16) and four cohort studies (14,15,17,18), were conducted to analyze the association between dietary acrylamide intake and risk of colorectal cancer. In these six studies, the summary RR for high versus low acrylamide intake was 0.94 (95% CI 0.85-1.04, P for trend=0.65) (10). In our study, the risk estimates have been consistently close to 1.00 for the overall and subgroup analyses.

Although green tea may lower the risks of esophageal, gastric, and colorectal cancer (35), the exposure to acrylamide from green tea is found to contribute substantially to the total dietary acrylamide exposure in Japan. Given that green tea is specific to the Japanese population, we conducted a stratified analysis to compare HRs between drinkers and non-drinkers. The results did not alter conclusions regarding the associations between

1 acrylamide intake and the cancers studied. As another common main source of acrylamide
2 both in Japan and Western countries, coffee was associated with decreased risk of
3 colorectal cancer (36). However, no preventive or causative effect was observed in our
4 study between coffee consumption and colorectal cancer (Supplementary Table S3).

5 We did not observe an overall association between dietary acrylamide intake and
6 risk of digestive organ cancer in this study. A previous study based on the JPHC Study
7 showed that dietary acrylamide intake was also not associated with breast cancer (37). In
8 our study, the average acrylamide intake was 6.8 $\mu\text{g}/\text{day}$, which is lower than 21.7 $\mu\text{g}/\text{day}$
9 of the Netherlands Cohort Study (NLCS) on diet and cancer and 26.2 $\mu\text{g}/\text{day}$ of the
10 European Prospective Investigation into Cancer and Nutrition (EPIC) (14). Although
11 there are differences in average acrylamide intake between Japanese and Western
12 populations, results of lack of association between dietary acrylamide intake and risk of
13 esophageal, gastric, or colorectal cancer are common worldwide. This indicates that there
14 might be no substantial difference in sensitivity for acrylamide intake between Asian and
15 Western populations.

16 This study has several strengths. It has a prospective cohort study design. Recall bias
17 in exposure was avoided since the data were collected before the diagnosis of cancer.
18 Participants were selected from the general population, and the sample size was large.
19 Moreover, the proportion of cases identified by death certificate only (DCO) was 7.1%
20 for esophageal cancer, 4.3% for gastric cancer, and 2.8% for colorectal cancer. Thus, the
21 cancer registries used in this study were of sufficient quality.

22 There are some limitations in this study. First, the FFQ has its own limitations, as
23 discussed elsewhere (38). However, the FFQ is the only feasible way to assess dietary
24 acrylamide intake over a long period of time in large-scale epidemiological studies.

1 Acrylamide levels vary greatly among foods, which may lead to misclassification of
2 dietary acrylamide intake. The energy-adjusted correlation coefficients of dietary
3 acrylamide intake from the FFQ and 28-day DRs ranged from 0.34 to 0.48 (25).
4 Furthermore, acrylamide values were estimated based on foods in the 2000s, which may
5 not completely represent foods in the 1990s. Compared with those in the 1990s, the
6 proportion of beverages was lower but the proportion of vegetables was higher in 2012
7 (Food Safety Commission of Japan. Study on estimate of acrylamide intake from food;
8 interim report. Food Safety Commission of Japan **2016**;
9 <https://www.fsc.go.jp/fsciis/technicalResearch/show/cho99920151507>). Second,
10 esophageal cancer risk factors may differ depending on squamous cell carcinoma or
11 adenocarcinoma. Due to the small number of esophageal cancer cases, it was difficult to
12 discuss what was the frequency of each event, and the statistical power of the stratified
13 analysis might have been affected; hence, the finding should be interpreted with caution.
14 Lastly, although possible confounding factors had been adjusted for in the analysis, other
15 unknown confounding factors may have impacts on the results. In conclusion, this study
16 demonstrated that dietary acrylamide intake was not associated with increased risk of
17 esophageal, gastric, or colorectal cancer among the Japanese population, in both overall
18 and stratified analyses.

19

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References

1. Sobel W, Bond GG, Parsons TW, Brenner FE. Acrylamide cohort mortality study. *Br J Ind Med* **1986**;43:785-8.
2. Collins JJ, Swaen GM, Marsh GM, Utidjian HM, Caporossi JC, Lucas LJ. Mortality patterns among workers exposed to acrylamide. *J Occup Med* **1989**;31:614-7.
3. Marsh GM, Lucas LJ, Youk AO, Schall LC. Mortality patterns among workers exposed to acrylamide: 1994 follow up. *Occup Environ Med* **1999**;56:181-90.
4. Marsh GM, Youk AO, Buchanich JM, Kant IJ, Swaen G. Mortality patterns among workers exposed to acrylamide: updated follow up. *J Occup Environ Med* **2007**;49:82-95.
5. Swaen GM, Haidar S, Burns CJ, Bodner K, Parsons T, Collins JJ, *et al.* Mortality study update of acrylamide workers. *Occup Environ Med* **2007**;64:396-401.
6. International Agency for Research on Cancer. Monographs on the evaluation of carcinogen risk to humans: some industrial chemicals. Lyon: International Agency for Research on Cancer; 1994.
7. Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem* **2002**;50:4998-5006.
8. Besaratinia A, Pfeifer GP. A review of mechanisms of acrylamide carcinogenicity. *Carcinogenesis* **2007**;28:519-28.
9. Shipp A, Lawrence G, Gentry R, McDonald T, Bartow H, Bounds J, *et al.* Acrylamide: review of toxicity data and dose-response analyses for cancer and noncancer effects. *Crit Rev Toxicol* **2006**;36:481-608.
10. Pelucchi C, Bosetti C, Galeone C, La Vecchia C. Dietary acrylamide and cancer

- 1 risk: An updated meta - analysis. Int J Cancer **2015**;136:2912-22.
- 2 11. Pelucchi C, Galeone C, Levi F, Negri E, Franceschi S, Talamini R, *et al.* Dietary
3 acrylamide and human cancer. Int J Cancer **2006**;118:467-71.
- 4 12. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA.
5 Dietary acrylamide intake is not associated with gastrointestinal cancer risk. J
6 Nutr **2008**;138:2229-36.
- 7 13. Lin Y, Lagergren J, Lu Y. Dietary acrylamide intake and risk of esophageal cancer
8 in a population - based case - control study in Sweden. Int J Cancer
9 **2011**;128:676-81.
- 10 14. Lujan-Barroso L, González CA, Slimani N, Obón-Santacana M, Ferrari P,
11 Freisling H, *et al.* Dietary intake of acrylamide and esophageal cancer risk in the
12 European Prospective Investigation into Cancer and Nutrition cohort. Cancer
13 Causes Control **2014**;25:639-46.
- 14 15. Hirvonen T, Kontto J, Jostoi M, Valsta L, Peltonen K, Pietinen P, *et al.* Dietary
15 acrylamide intake and the risk of cancer among Finnish male smokers. Cancer
16 Causes Control **2010**;21:2223-9.
- 17 16. Mucci LA, Dickman PW, Steineck G, Adami HO, Augustsson K. Dietary
18 acrylamide and cancer of the large bowel, kidney, and bladder: absence of an
19 association in a population-based study in Sweden. Br J Cancer **2003**;88:84-9.
- 20 17. Mucci LA, Adami HO, Wolk A. Prospective study of dietary acrylamide and risk
21 of colorectal cancer among women. Int J Cancer **2006**;118:169-73.
- 22 18. Larsson SC, Åkesson A, Bergkvist L, Wolk A. Dietary acrylamide intake and
23 risk of colorectal cancer in a prospective cohort of men. Eur J Cancer
24 **2009**;45:513-6.

- 1 19. Kotemori A, Ishihara J, Zha L, Liu R, Sawada N, Iwasaki M, *et al.* Dietary
2 acrylamide intake and the risk of endometrial or ovarian cancers in Japanese
3 women. *Cancer Sci* **2018**;109:3316.
- 4 20. Tsugane S, Sobue T. Baseline survey of JPHC study design and participation rate.
5 *J Epidemiol* **2001**;11 (Suppl 6):S24-9.
- 6 21. Tsugane S, Sasaki S, Kobayashi M, Tsubono Y, Akabane M. Validity and
7 reproducibility of the self-administered food frequency questionnaire in the JPHC
8 Study Cohort I: study design, conduct and participant profiles. *J Epidemiol*
9 **2003**;13 (Suppl 1):S2-12.
- 10 22. Ishihara J, Sobue T, Yamamoto S, Yoshimi I, Sasaki S, Kobayashi M, *et al.*
11 Validity and reproducibility of a self-administered food frequency questionnaire
12 in the JPHC Study Cohort II: study design, participant profile and results in
13 comparison with Cohort I. *J Epidemiol* **2003**;13 (Suppl 1):S134-47.
- 14 23. Ishihara J, Inoue M, Kobayashi M, Tanaka S, Yamamoto S, Iso H, *et al.* Impact of
15 the revision of a nutrient database on the validity of a self-administered food
16 frequency questionnaire (FFQ). *J Epidemiol* **2006**;16:107-16.
- 17 24. Resource Council, Science and Technology Agency, the Government of Japan.
18 Standard tables of food composition in Japan, the fifth revised edition. Tokyo,
19 Japan: Printing Bureau, Ministry of Finance **2002**.
- 20 25. Kotemori A, Ishihara J, Nakadate M, Sawada N, Iwasaki M, Sobue T, *et al.*
21 Validity of a self-administered food frequency questionnaire for the estimation of
22 acrylamide intake in the Japanese population: the JPHC FFQ Validation Study. *J*
23 *Epidemiol* **2017**.
- 24 26. FAO/WHO. Health implications of acrylamide in food. Report of a Joint

1 FAO/WHO Consultation. Geneva: FAO/WHO **2002**.

2 27. Yoshida M, Ono H, Ohnishi Kameyama M, Chuda Y, Yada H, Kobayashi H, *et al*.
 3 Determination of acrylamide in processed foodstuffs in Japan. Nippon Shokuhin
 4 Kagaku Kogaku Kaishi **2002**;49:822-5.

5 28. Takatsuki S, Nemoto S, Sasaki K, Maitani T. Production of acrylamide in
 6 agricultural products by cooking. J Food Hyg Soc Japan **2004**;45:44-8.

7 29. Mizukami Y, Kohata K, Yamaguchi Y, Hayashi N, Sawai Y, Chuda Y, *et al*.
 8 Analysis of acrylamide in green tea by gas chromatography– mass spectrometry.
 9 J Agric Food Chem **2006**;54:7370-7.

10 30. Yoshida M, Miyoshi K, Horibata K, Mizukami Y, Takenaka M, Yasui A.
 11 Estimation of acrylamide intake from cooked rice in Japan. Nippon Shokuhin
 12 Kagaku Kogaku Kaishi **2011**;58:525-30.

13 31. Enzinger PC, Mayer RJ. Esophageal cancer. N Engl J Med **2003**;349:2241-52.

14 32. Colquhoun A, Arnold M, Ferlay J, Goodman K, Forman D, Soerjomataram I.
 15 Global patterns of cardia and non-cardia gastric cancer incidence in 2012. Gut
 16 **2015**;64:1881-8.

17 33. Wei EK, Giovannucci E, Wu K, Rosner B, Fuchs CS, Willett WC, *et al*.
 18 Comparison of risk factors for colon and rectal cancer. Int J Cancer **2004**;108:433-
 19 42.

20 34. Schettgen T, Rossbach B, Kütting B, Letzel S, Drexler H, Angerer J.
 21 Determination of haemoglobin adducts of acrylamide and glycidamide in
 22 smoking and non-smoking persons of the general population. Int J Hyg Environ
 23 Health **2004**;207:531-9.

24 35. Jankun J, Selman SH, Swiercz R, Skrzypczak-Jankun E. Why drinking green tea

- 1 could prevent cancer. *Nature* **1997**;387:561.
- 2 36. Nkondjock A. Coffee consumption and the risk of cancer: an overview. *Cancer*
- 3 *Lett* **2009**;277:121-5.
- 4 37. Kotemori A, Ishihara J, Zha L, Liu R, Sawada N, Iwasaki M, *et al.* Dietary
- 5 acrylamide intake and risk of breast cancer: The Japan Public Health Center -
- 6 based Prospective Study. *Cancer Sci* **2018**;109:843-53.
- 7 38. Riboldi BP, Vinhas ÁM, Moreira JD. Risks of dietary acrylamide exposure: a
- 8 systematic review. *Food Chem* **2014**;157:310-22.
- 9

Table 1. Characteristics of participants (n = 87,628) for esophageal, gastric and colorectal cancer analysis

Characteristics	Quintiles of energy-adjusted acrylamide intake												P-value ^a			
	Quintile 1			Quintile 2			Quintile 3			Quintile 4				Quintile 5		
Number of participants	17,526			17,526			17,525			17,526			17,525			
Number of men (%)	23.1			20.3			19.0			18.5			19.1			
Number of women (%)	17.3			19.8			20.9			21.3			20.8			
Dietary variables																
Acrylamide intake																
Range, $\mu\text{g}/\text{d}$	0.0	-	3.8	3.8	-	5.3	5.3	-	6.9	6.9	-	9.4	9.4	-	63.5	
Mean and SD, $\mu\text{g}/\text{d}$	2.8	±	0.8	4.6	±	0.4	6.0	±	0.5	8.0	±	0.7	12.7	±	3.6	
Mean and SD, $\mu\text{g} \cdot \text{kg body weight}^{-1} \cdot \text{d}^{-1}$	0.05	±	0.04	0.08	±	0.06	0.11	±	0.10	0.15	±	0.12	0.24	±	0.28	
Coffee, g/d	27.6	±	43.3	65.0	±	69.6	103.0	±	96.1	169.8	±	147.2	337.6	±	317.7	<0.001
Green tea, g/d	255.3	±	290.5	422.8	±	383.9	517.5	±	420.9	596.4	±	470.1	859.5	±	761.1	<0.001
Alcohol intake, g/d	161.1	±	256.5	116.5	±	202.5	93.4	±	180.4	81.8	±	164.8	61.1	±	133.5	<0.001
Vegetables, g/d	173.2	±	119.7	211.2	±	126.0	226.3	±	131.7	231.6	±	135.5	229.5	±	146.8	<0.001
Potato, g/d	8.5	±	8.1	14.5	±	12.1	18.2	±	15.0	20.1	±	18.2	21.8	±	26.8	<0.001
Fruit, g/d	169.2	±	165.8	206.6	±	161.4	221.1	±	167.3	221.5	±	166.0	208.1	±	166.9	<0.001
Meat, g/d	58.5	±	46.0	58.0	±	38.4	56.9	±	36.4	56.8	±	35.3	55.5	±	35.4	<0.001
Fish, g/d	85.3	±	59.5	89.5	±	51.3	88.7	±	48.2	86.6	±	48.2	81.1	±	47.5	<0.001
Dairy food, g/d	198.0	±	229.0	183.6	±	186.2	178.5	±	169.9	171.1	±	163.6	152.2	±	156.3	<0.001
Soy food, g/d	92.5	±	100.8	90.7	±	77.8	87.6	±	69.6	84.4	±	67.1	79.3	±	62.4	<0.001
Biscuits, g/d	0.6	±	1.0	1.3	±	1.6	2.0	±	2.5	3.2	±	4.1	5.9	±	9.5	<0.001
Total energy intake, kcal/d	1984.5	±	653.7	2025.6	±	616.9	2017.0	±	610.4	2021.4	±	608.5	1920.3	±	606.9	<0.001

Non-dietary variables

Age at 5-year follow-up study, y	58.1	±	7.5	57.6	±	7.8	57.3	±	7.9	56.7	±	8.1	55.8	±	8.0	<0.001
Body mass index, kg/m ²	23.7	±	3.1	23.6	±	3.0	23.6	±	3.0	23.5	±	3.0	23.4	±	3.0	<0.001
Smoking status, %																
Never	59.5			64.4			65.1			64.2			59.3			
Former	10.4			9.4			9.2			8.2			8.0			
Current	25.0			21.9			21.0			23.2			28.4			<0.001
Missing	5.0			4.4			4.7			4.5			4.3			
Pack years, for former and current smokers	34.7	±	25.8	34.4	±	19.6	34.5	±	19.8	35.8	±	24.8	37.8	±	21.5	<0.001
Physical activity (METs)	36.0	±	10.1	37.4	±	9.6	37.5	±	9.5	37.6	±	9.4	37.5	±	9.5	<0.001

Values are mean ± SD, or percentages.

^a Kruskal-Wallis test for continuous variables and chi-square test for categorical variables.

Table 2. Hazard ratios (95% confidence intervals) for esophageal cancer according to quintiles of acrylamide intake.

		Quintiles of energy-adjusted acrylamide intake										<i>P</i> for trend
		Total	Quintile 1		Quintile 2		Quintile 3		Quintile 4		Quintile 5	
		HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	
Esophageal cancer												
Number of subjects	87,628											
Person-years	1,354,648											
Number of cases	391											
Age- and area-adjusted mode 1 ^a		1.00	(Reference)	0.90	(0.67-1.21)	0.93	(0.69-1.25)	0.97	(0.72-1.31)	0.67	(0.48-0.94)	0.078
Multivariable model ^b		1.00	(Reference)	1.03	(0.76-1.38)	1.14	(0.84-1.54)	1.19	(0.87-1.62)	0.84	(0.59-1.19)	0.813
Multivariable model (excluding cases <3 y) ^b		1.00	(Reference)	0.98	(0.71-1.36)	1.17	(0.84-1.62)	1.21	(0.87-1.68)	0.87	(0.60-1.26)	0.960
Esophageal squamous cell carcinoma												
Number of cases	305											
Age- and area-adjusted model ^a		1.00	(Reference)	0.98	(0.70-1.37)	1.01	(0.72-1.41)	0.95	(0.67-1.35)	0.71	(0.48-1.04)	0.126
Multivariable model ^c		1.00	(Reference)	1.11	(0.79-1.55)	1.24	(0.88-1.74)	1.18	(0.83-1.69)	0.93	(0.62-1.38)	0.963
Esophageal adenocarcinoma												
Number of cases	20											
Age- and area-adjusted model ^a		1.00	(Reference)	0.90	(0.24-3.38)	0.68	(0.16-2.89)	0.70	(0.17-2.99)	1.15	(0.32-4.11)	0.959
Multivariable model ^d		1.00	(Reference)	1.03	(0.27-3.90)	0.79	(0.18-3.42)	0.79	(0.18-3.45)	1.25	(0.34-4.59)	0.873

Abbreviations: 95% CI = 95% confidence intervals.

^a Age- and area-adjusted model adjusted for age (continuous), sex and area (10 public health center areas).^b Multivariable model additionally adjusted for: smoking status (never, former, current, missing), body mass index (<23.0, 23.0-24.9, 25.0-26.9, >=27.0 kg/m², missing), physical activity (continuous), intakes of alcohol (<150, ≥ 150 g/week), and energy-adjusted consumption of vegetables, fruits, and dairy (continuous).

^c Multivariable model additionally adjusted for: smoking status (never, former, current, missing), physical activity (continuous), intakes of alcohol (<150, \geq 150 g/week), and energy-adjusted consumption of vegetables, fruits, and dairy (continuous). BMI was not included since it is not a risk factor for ESCC.

^d Multivariable model additionally adjusted for: smoking status (never, former, current, missing), body mass index (<23.0, 23.0-24.9, 25.0-26.9, \geq 27.0 kg/m², missing), physical activity (continuous), and energy-adjusted consumption of vegetables, fruits, and dairy (continuous). Alcohol was not included since it is not a risk factor for EAC.

Table 3. Hazard ratios (95% confidence intervals) for gastric cancer according to quintiles of acrylamide intake.

		Quintiles of energy-adjusted acrylamide intake										<i>P</i> for trend
		Quintile 1		Quintile 2		Quintile 3		Quintile 4		Quintile 5		
	Total	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	
Gastric cancer												
Number of subjects	87,628											
Person-years	1,344,756											
Number of cases	2,218											
Age- and area-adjusted model ^a		1.00	(Reference)	0.92	(0.81-1.05)	1.04	(0.91-1.18)	1.02	(0.90-1.17)	0.92	(0.80-1.06)	0.756
Multivariable model ^b		1.00	(Reference)	0.92	(0.81-1.05)	1.03	(0.91-1.17)	1.01	(0.88-1.15)	0.90	(0.79-1.04)	0.551
Multivariable model (excluding cases <3 y) ^b		1.00	(Reference)	0.90	(0.79-1.04)	1.02	(0.88-1.17)	1.04	(0.90-1.20)	0.92	(0.79-1.07)	0.956
Cardia gastric cancer												
Number of cases	138											
Age- and area-adjusted model ^a		1.00	(Reference)	0.79	(0.44-1.40)	1.49	(0.90-2.45)	1.33	(0.78-2.24)	1.28	(0.74-2.21)	0.114
Multivariable model ^b		1.00	(Reference)	0.81	(0.46-1.45)	1.57	(0.95-2.62)	1.40	(0.82-2.39)	1.35	(0.77-2.37)	0.082
Non-cardia gastric cancer												
Number of cases	2080											
Age- and area-adjusted model ^a		1.00	(Reference)	0.93	(0.82-1.06)	1.01	(0.88-1.15)	1.01	(0.88-1.15)	0.90	(0.78-1.04)	0.466
Multivariable model ^c		1.00	(Reference)	0.93	(0.81-1.06)	1.00	(0.88-1.15)	0.99	(0.86-1.13)	0.88	(0.76-1.02)	0.293

Abbreviations: 95% CI = 95% confidence intervals.

^a Age- and area-adjusted model adjusted for age (continuous), sex and area (10 public health center areas).^b Multivariable model additionally adjusted for: smoking status (never, former, current, missing), body mass index (<23.0, 23.0-24.9, 25.0-26.9, ≥27.0 kg/m², missing), physical activity (continuous), intakes of alcohol (<150, ≥150 g/week), and energy-adjusted consumption of vegetables, fruits, and salted fish (continuous).

^c Multivariable model additionally adjusted for: smoking status (never, former, current, missing), physical activity (continuous), intakes of alcohol (<150, \geq 150 g/week), and energy-adjusted consumption of vegetables, fruits, and salted fish (continuous). BMI was not included since it is not considered a risk factor for NCGC.

Table 4. Hazard ratios (95% confidence intervals) for colorectal cancer according to quintiles of acrylamide intake.

		Quintiles of energy-adjusted acrylamide intake										<i>P</i> for trend
		Quintile 1		Quintile 2		Quintile 3		Quintile 4		Quintile 5		
	Total	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	
Colorectal cancer												
Number of subjects	87,628											
Person-years	1,342,251											
Number of cases	2,470											
Age- and area-adjusted model ^a		1.00	(Reference)	1.02	(0.91-1.15)	0.89	(0.78-1.00)	0.92	(0.81-1.04)	0.89	(0.78-1.01)	0.015
Multivariable model ^b		1.00	(Reference)	1.06	(0.94-1.19)	0.93	(0.82-1.05)	0.97	(0.85-1.10)	0.94	(0.83-1.08)	0.172
Multivariable model (excluding cases <3 y) ^b		1.00	(Reference)	1.07	(0.95-1.22)	0.96	(0.84-1.10)	0.95	(0.83-1.09)	0.97	(0.84-1.12)	0.258
Colon cancer												
Number of cases	1,721											
Age- and area-adjusted model ^a		1.00	(Reference)	0.98	(0.85-1.12)	0.87	(0.75-1.00)	0.87	(0.75-1.01)	0.83	(0.71-0.97)	0.005
Multivariable model ^b		1.00	(Reference)	1.01	(0.88-1.16)	0.91	(0.78-1.06)	0.92	(0.79-1.07)	0.89	(0.76-1.04)	0.068
Rectal cancer												
Number of cases	749											
Age- and area-adjusted model ^a		1.00	(Reference)	1.14	(0.92-1.42)	0.94	(0.74-1.18)	1.03	(0.82-1.30)	1.02	(0.81-1.30)	0.856
Multivariable model ^c		1.00	(Reference)	1.17	(0.94-1.45)	0.97	(0.76-1.22)	1.07	(0.84-1.35)	1.06	(0.83-1.35)	0.927

Abbreviations: 95% CI = 95% confidence intervals.

^a Age- and area-adjusted model adjusted for age (continuous), sex and area (10 public health center areas).^b Multivariable model additionally adjusted for: smoking status (never, former, current, missing), body mass index (<23.0, 23.0-24.9, 25.0-26.9, ≥27.0 kg/m², missing), physical activity (continuous), intakes of alcohol (<150, ≥150 g/week), and energy-adjusted consumption of vegetables, fruits, meat, and dairy (continuous).

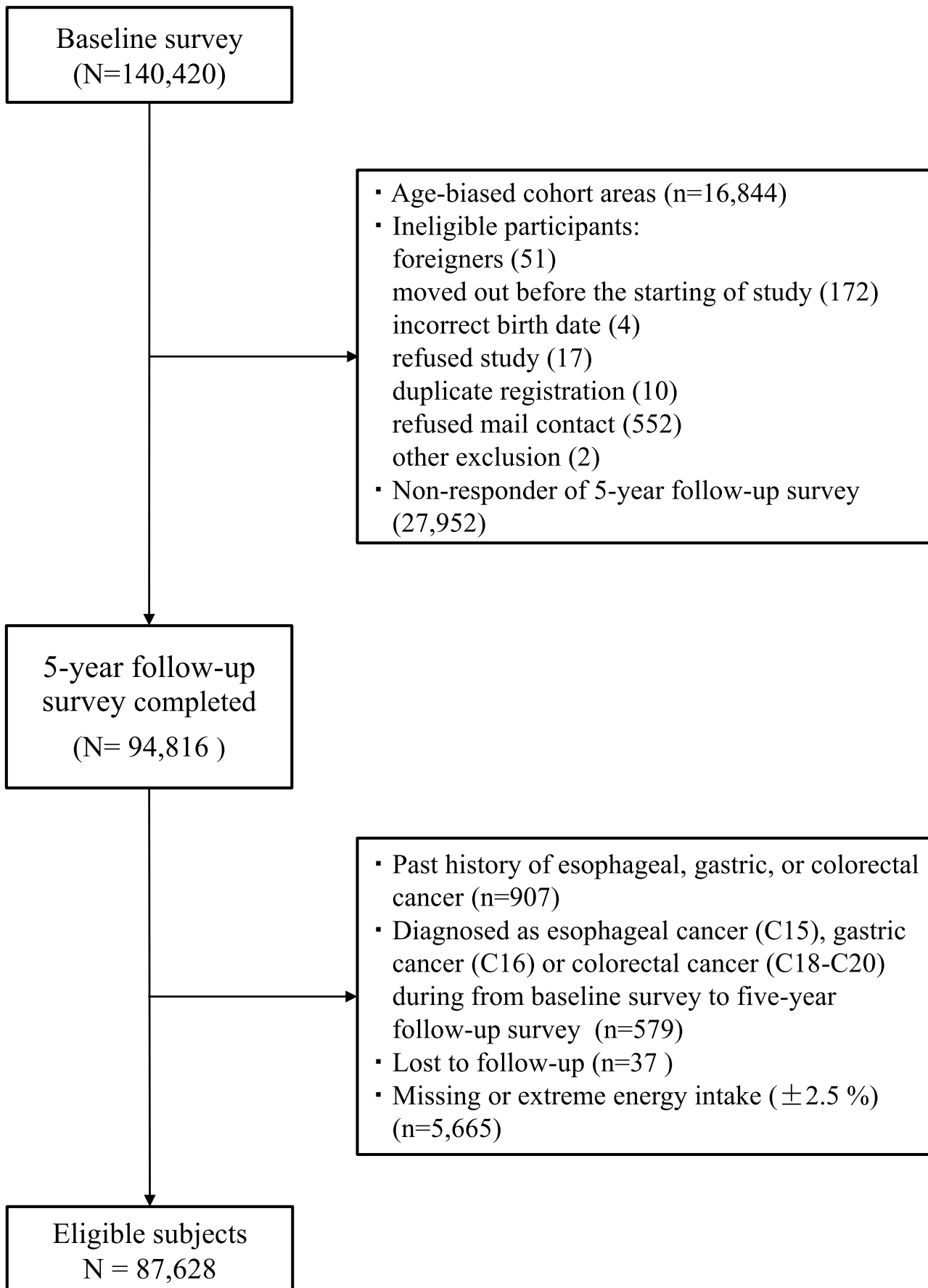
^c Multivariable model additionally adjusted for: smoking status (never, former, current, missing), body mass index (<23.0, 23.0-24.9, 25.0-26.9, \geq 27.0 kg/m², missing), intakes of alcohol (<150, \geq 150 g/week), and energy-adjusted consumption of vegetables, fruits, meat, and dairy (continuous). Physical activity was not included since it is not considered a protective factor for rectal cancer.

1 **Figure Legends**

2 Figure 1. Flow-chart of study participants: JPHC Study.

3 Figure 1 is a flow-chart describing the analysis sample of the JPHC Study (N=140,420),

4 exclusion criteria, and eligible analysis subjects (N=87,628).



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Dietary Acrylamide Intake and Risk of Esophageal, Gastric, and Colorectal Cancer: The Japan Public Health Center-based Prospective Study

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Dietary intake of acrylamide and esophageal cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort

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Abstract

Purpose The relation between dietary acrylamide intake and esophageal cancer (EC) risk, including esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC), has not been consistent. We evaluated the association between dietary acrylamide intake and

EAC, ESCC, and overall EC in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. **Methods** Multivariate Cox proportional hazards models were used to estimate the HR and 95 % confidence interval (95 % CI). Since nonlinear relations were observed, HRs were displayed for quartiles of acrylamide intake in µg per day.

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Results After a mean follow-up of 11 years, 341 EC were identified, 142 of which were EAC, 176 ESCC, and 23 other histological types or not specified. An increase in EC risk was observed in the second and third quartiles (HR_{Q2vsQ1} 1.75, 95 % CI 1.12–2.74; HR_{Q3vsQ1} 1.66, 95 % CI 1.05–2.61), but not in the fourth quartile, and there was no evidence for a linear dose–response trend. HRs were similarly elevated but not statistically significant when ESCC and EAC were analyzed separately, due to the small number of cases observed. No associations were observed when quartiles were based on energy-adjusted acrylamide intake.

Conclusions In the EPIC cohort, an association between estimated dietary acrylamide intake and an increased risk of developing EC was observed in the middle quartiles but not in the highest quartile; however, results from other larger cohorts or consortia, and results from biomarker studies, might add to the evidence provided by this analysis, suggesting that acrylamide is not an important risk factor for EC.

Keywords Esophageal cancer · Esophageal squamous cell carcinoma · Esophageal adenocarcinoma · Acrylamide intake · Cohort · Nutrition

Introduction

In 1994, the International Agency for Research on Cancer (IARC) classified acrylamide as ‘probably carcinogenic’ to humans based on animal studies and evidence in humans

[1]. In 2002, acrylamide was discovered at relatively high concentrations in some foods. Acrylamide in foods is formed through the Maillard reaction during high temperature ($>120^{\circ}\text{C}$) cooking, primarily in foods of plant origin such as potatoes, breads, and cereals [2]. The main determinants of dietary intake of acrylamide in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort based on a 24-h dietary recall (24HDR) were bread, crispbread, rusks, coffee, potatoes, cakes, biscuits, and cookies [3].

The evidence for an association between estimated acrylamide intake based on dietary questionnaires (DQs) and cancer risk has been inconsistent in epidemiological studies [2]. Two case–control studies [4, 5] and one case–cohort study [6] have evaluated the association between estimated acrylamide intake and esophageal cancer (EC) including the histological subtypes esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC), but only the Swedish case–control study ($n = 594$ EC, including $n = 189$ EAC and $n = 167$ ESCC cases) [4] observed an increase in overall EC risk. This association was stronger among overweight or obese persons ($n = 268$ EC). The Netherlands Cohort Study on Diet and Cancer (NLCS) ($n = 216$ EC, including $n = 115$ EAC and $n = 90$ ESCC cases) observed an increase in risk per 10 μg increment of acrylamide intake per day in obese persons, but this observation was based on only 20 EC cases, including 14 EAC [6].

The aim of the present study was to assess whether pre-diagnostic dietary acrylamide intake levels based on DQs are associated with the risk of developing, ESCC, EAC, and overall EC in the EPIC cohort.

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Materials and methods

EPIC is a multicenter cohort study which recruited participants from 23 centers located in ten European countries. At baseline, information was collected on lifestyle factors, sociodemographic characteristics, medical history, and the usual diet over the previous 12 months using validated country-specific DQs [7]. To estimate the average daily intake of acrylamide, we matched the DQ food intake data to a harmonized acrylamide database, which we compiled from the EU monitoring database of acrylamide levels in food maintained by the European Community Institute for Reference Materials and Measurements (IRMM) and other sources [3, 8]. Case definitions for EC, EAC, and ESCC have been previously published [9].

Proportional hazards modeling was used to estimate HRs and 95 % confidence interval (95 % CI) for estimated dietary acrylamide intake and EC, ESCC, and EAC risk. Age at recruitment was used as entry time and age at first EC for cases or age at censoring time for non-cases were used as exit time. EC, ESCC, and EAC multivariate models were stratified by country to control for country effects (recruitment strategies, questionnaire design, and follow-up procedures). Age at recruitment (1-year categories) was used as the primary time variable. All models were adjusted for total energy intake, sex, cigarette smoking status, number of cigarettes, time since quitting, intakes of total fruits and processed meat. ESCC and EAC multivariate models were also adjusted for body mass index (BMI), and EC and ESCC for alcohol intake [9]. Different risk factors in the multivariate models were used because

of etiologic heterogeneity between ESCC and EAC [10]. Schoenfeld residuals were used to assess the proportional hazard assumption [11]. Restricted cubic splines (RCS) with 3–5 knots were used to explore dose–response linearity [12]. Akaike information criterion (AIC) was used to select the best representation of the relation between dietary acrylamide intake and EC, also comparing with the linear model. The minimum AIC was found with the RCS with four knots (5, 35, 65, and 95th percentiles of the distribution of dietary acrylamide intake). Since the relation was not linear, different transformations of the estimated acrylamide intake variable (such as natural logarithm and root square) were evaluated; however, the relation remained nonlinear (with higher estimated intakes showing weaker associations with EC risk) (data not shown). Thus, results for continuous variables of estimated acrylamide intake were not displayed. HRs are presented as the change in cancer risk for each quartile relative to the lowest quartile of estimated acrylamide intake (quartiles were calculated based on the full cohort). Estimations of acrylamide intake were corrected using the residual method to control for the effect of total energy intake and to reduce the impact of measurement error in DQs [13, 14]. Quartiles of acrylamide intake were also based on energy-adjusted intake in both men and women in the EPIC cohort.

Effect-measure modification by smoking status (smokers vs. never smokers + ≥ 20 years quitters), sex, BMI (normal vs. overweight or obese), and alcohol intake was evaluated using a likelihood ratio test (LRT). Since some risk factors differ for ESCC and EAC, effect-measure modification was evaluated separately for EC, ESCC, and

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EAC. A positive association between obesity and EAC has been demonstrated [9, 15], and EPIC [9] and other studies [16] have observed that obesity may be inversely related to ESCC. Since the direction of risk with obesity is different for EAC and ESCC, potential effect-measure modification between estimated acrylamide intake and body weight (BMI <25 vs. BMI ≥25) was evaluated for ESCC and EAC separately. Since alcohol drinking is a risk factor for ESCC, effect-measure modification by alcohol intake was only evaluated for ESCC, comparing low intakes (<12 g/day for men and <6 g/day for women) versus higher intakes. Finally, due to the low number of female EAC cases ($n = 28$), effect-measure modification by sex was evaluated only for EC and ESCC. Tertiles and quartiles of estimated acrylamide intake were used to evaluate effect-measure modification for ESCC, EAC, and EC.

Sensitivity analyses, excluding EC cases and censoring participants during the first 2 years of follow-up, were carried out to evaluate the possible influence of prior diseases on dietary habits. Further, because smoking is an important determinant of acrylamide exposure, analyses were performed in never smokers and those individuals who had quit at least 20 years before being enrolled in the EPIC cohort.

Results

After a mean follow-up of 11 years, 341 ECs cases were identified including 142 EACs, 176 ESCCs, and 23 that were other histological types or were not specified. At baseline, the mean of estimated dietary acrylamide intake based on DQs in EPIC was 26.22 µg/day. More details on the distribution of dietary acrylamide intake in the EPIC cohort centers have been previously published [8]. Individuals with the lowest estimated acrylamide intake values had the highest intakes of total fruits (Table 1), and participants with the highest quartile of acrylamide intake had the highest intakes of processed meat, alcohol, and total

energy and were more likely to be current smokers at baseline (Table 1).

For overall EC, participants with estimated acrylamide intakes ranging from 15.7 to 23.3 µg/day (second quartile) had a 75 % increased risk of developing EC compared with participants in the lowest quartile of estimated intake (0–15.6 µg/day), while participants with a range of estimated intake from 23.4 to 30.7 µg/day (third quartile) had a 66 % higher risk of developing cancer (Table 2). Individuals in the highest quartile of estimated intake (34.2–261.4 µg/day) were not at statistically significantly increased risk of EC, and further, no linear dose–response trends were observed for overall EC. The analysis was repeated using the sex-specific quintile cut-points defined in the NLCS study, and the association between estimated acrylamide intake and EC was statistically significant in the third and fourth quintiles (HR_{Q3vsQ1} 1.87, 95 % CI 1.01–3.47; HR_{Q4vsQ1} 2.17, 95 % CI 1.20–3.92), but not in the second or fifth (HR_{Q2vsQ1} 1.70, 95 % CI 0.88–3.26; HR_{Q5vsQ1} 1.67, 95 % CI 0.91–3.12) (data not in tables).

When we analyzed ESCC and EAC separately, the same pattern was observed, but none of the HR estimates were statistically significant (Table 2). When the analysis was restricted to never smokers and former smokers who had quit at least 20 years before baseline, similar results were observed (Table 2). Quartiles based on energy-adjusted acrylamide intake showed no association with EC, even when EC was evaluated by histological subtypes (Table 2). Further, there was no evidence for effect-measure modification of the relation between acrylamide intake and EC risk by smoking status, sex, BMI, or alcohol intake (all LRT p values >0.06) (data not shown).

Discussion

We did not observe convincing evidence that estimated acrylamide intake based on DQs is associated with esophageal cancer risk in the EPIC cohort. While we detected elevated and significant HRs for estimated acrylamide intake in the second and third quartiles, we did not observe a statistically significant increase risk in the fourth quartile, and there was no evidence for a linear dose–response trend. When the analysis was performed using quartiles based on energy-adjusted acrylamide intake, none of the results were statistically significant. Similar patterns were seen when results were analyzed by histological subtype (ESCC or EAC), but none of the HRs were statistically significant. Because smoking is an important determinant of acrylamide exposure, analyses were carried out in never smokers and former smokers who had quit at least 20 years before baseline, and similar patterns were observed.

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Table 1 Baseline characteristics by quartiles of estimated dietary acrylamide intake based on dietary questionnaires

	Cohort	Total estimated acrylamide intake (µg/day)			
		Q1: 0–15.6	Q2: 15.7–23.3	Q3: 23.4–34.1	Q4: 34.2–261.4
Estimated acrylamide median, µg/day ^a	23.3 (15.7–34.0)	11.3 (8.3–13.6)	19.4 (17.55–21.28)	27.9 (25.5–30.7)	37.9 (51.4–43.2)
Age at recruitment ^a	51.5 (45.1–58.2)	51.5 (45.6–58.2)	50.7 (44.9–57.9)	51.4 (44.6–58.4)	52.2 (45.0–58.3)
Sex (%)					
Male	29.8	19.4	23.0	31.2	47.7
Female	70.2	80.6	79.0	68.8	52.3
Cigarette smoking status (%)					
Never	48.9	53.3	51.9	48.6	41.8
Former	26.6	24.2	25.2	27.4	29.8
Current	22.4	20.2	20.4	22.1	27.1
Unknown	2.0	2.3	2.6	1.9	1.3
Number of cigarettes (c/day) ^{a, b}	14.0 (10.0–20.0)	11.0 (6.0–20.0)	10.0 (10.0–20.0)	15.0 (10.0–20.0)	15.0 (10.0–20.0)
Time since quitting smoking, y ^{a, c}	14.0 (6.5–22.0)	13.0 (6.5–20.5)	14.5 (6.5–22.0)	14.5 (6.5–23.0)	14.5 (6.5–23.0)
BMI, kg/m ^{2a}	24.8 (22.4–27.8)	24.9 (22.4–28.0)	24.5 (22.1–27.5)	24.8 (22.3–27.7)	25.1 (22.7–27.9)
Alcohol at recruitment, g/day ^a	5.3 (0.9–14.9)	3.0 (0.3–12.3)	3.9 (0.8–12.0)	6.2 (1.3–15.6)	8.4 (2.0–19.5)
Total fruits, g/day ^{a, d}	200.3 (111.4–321.9)	245.3 (137.2–367.7)	201.0 (112.8–320.9)	189.2 (106.0–306.0)	173.1 (98.1–282.2)
Processed meat, g/day ^a	24.3 (10.5–43.9)	18.7 (7.9–34.3)	25.2 (11.0–43.9)	26.4 (11.6–47.2)	28.4 (12.7–50.4)
Total energy intake, Kcal ^a	1,996 (1,630–2,435)	1,700 (1,384–2,098)	1,856 (1,560–2,212)	2,069 (1,742–2,462)	2,381 (1,999–2,832)

^a Median (25–75th percentile)

^b Only for current smokers

^c Only for former smokers

^d Total fruits: fruits, nuts, and seeds

The only other prospective study to analyze the association between estimated dietary acrylamide intake and EC was in the NLCS which detected no overall associations between acrylamide intake and EC, ESCC, or EAC risk; however, in a subsample of obese participants (20 EC and 14 EAC cases), some elevated risk estimates were observed. In EPIC, no statistically significant associations between estimated acrylamide intake and EAC or ESCC risk were observed in overweight or obese participants (ESCC: HR_{T2vsT1} 1.47, 95 % CI 0.74–2.94; HR_{T3vsT1} 1.08, 95 % CI 0.50–2.33, and EAC: HR_{T2vsT1} 1.33, 95 % CI 0.64–2.77; HR_{T3vsT1} 1.23, 95 % CI 0.56–2.67). When we re-analyzed the relation between estimated acrylamide intake and EC using the same sex-specific cut-points used in the NLCS study, we observed significant HRs in the third and fourth quintiles (which had similar estimated intake ranges to our second and third quartiles), but not in the fifth quintile. The Swedish case-control study reported a positive association with overall EC (OR_{Q4vsQ1} 1.23, 95 % CI 1.02–1.75), and in overweight or obese persons (OR_{Q4vsQ1} 1.88, 95 % CI 1.06–3.34). The Italian case-control study ($n = 395$ EC cases) reported no association

between estimated dietary acrylamide intake and overall EC.

Results presented for overall EC should be interpreted with caution because while ESCC and EAC share some risk factors, they are also known to have distinct etiologies [10]. ESCC is usually located in the middle of the esophagus and the principal risk factors are tobacco smoking and high levels of alcohol consumption [10, 15]. Nearly, all EACs are located in the distal one-third of the esophagus, and Barrett's esophagus [17], tobacco smoking, and obesity are major risk factors [15]. Both cancers share processed meat as a possible risk factor [18–20], and fruits and vegetables [15, 21, 22] have generally shown protective associations for both. Thus, as estimated dietary acrylamide associations in both subtypes gave similar HRs estimates, we posited that estimated acrylamide effects (if they exist) would be similar among histological subtypes; thus, overall results for combined histologies were also presented.

In light of the differences mentioned between the studies on acrylamide and EC, it is also worth noting that the three published studies and EPIC used different acrylamide

Table 2 Hazard ratios and 95 % CI for estimated dietary acrylamide intake and EC, ESCC, and EAC in the EPIC cohort

	EC			ESCC			EAC		
	Cases	PY ^a	HR (95 % CI) ^b	Cases	PY ^a	HR (95 % CI) ^c	Cases	PY ^a	HR (95 % CI) ^d
<i>Full cohort</i>									
Quartiles (µg/day)									
Estimated acrylamide intake (µg/day)									
Q1 (0–15.6)	32	1,299,314	reference	23	1,299,314	reference	8	1,299,314	reference
Q2 (15.7–23.3)	76	1,298,899	1.75 (1.12–2.74)	44	1,298,899	1.63 (0.94–2.80)	26	1,298,899	1.97 (0.83–4.68)
Q3 (23.4–30.7)	104	1,323,954	1.66 (1.05–2.61)	53	1,323,954	1.52 (0.86–2.68)	44	1,323,954	2.01 (0.85–4.74)
Q4 (34.2–261.4)	129	1,340,783	1.41 (0.86–2.71)	56	1,340,783	1.23 (0.65–2.29)	64	1,340,783	1.82 (0.74–4.47)
Energy-adjusted acrylamide intake (µg/day)									
Q1 (0–17.7)	51	1,306,465	reference	34	1,306,465	reference	13	1,306,465	reference
Q2 (17.8–24.4)	67	1,289,531	1.12 (0.75–1.68)	41	1,289,531	1.19 (0.72–1.98)	23	1,289,531	1.29 (0.61–2.75)
Q3 (24.5–33.2)	96	1,327,405	1.11 (0.75–1.66)	43	1,327,405	0.95 (0.56–1.61)	48	1,327,405	1.59 (0.78–3.25)
Q4 (33.2–244.6)	127	1,339,549	1.04 (0.69–1.56)	58	1,339,549	0.99 (0.56–1.71)	58	1,339,549	1.24 (0.60–2.55)
<i>Never smokers + ≥ 20 years quitters</i>									
Quartiles (µg/day)									
Estimated acrylamide intake (µg/day) ^e									
Q1 (0–15.6)	8	781,180	reference	7	1,045,423	reference	6	1,045,423	reference
Q2 (15.7–23.3)	24	784,146	1.97 (0.85–4.55)	22	1,034,795	2.03 (0.82–5.06)	25	1,034,795	2.11 (0.81–5.50)
Q3 (23.4–30.7)	50	766,646	2.77 (1.21–6.33)	20	94,676	1.54 (0.54–4.39)	33	94,676	1.79 (0.67–5.04)
Q4 (34.2–261.4)	37	695,007	1.66 (0.66–4.15)	–	–	–	–	–	–
Energy-adjusted acrylamide intake (µg/day) ^f									
Q1 (0–17.7)	13	763,926	reference	9	1,034,292	reference	7	1,034,292	reference
Q2 (17.8–24.4)	24	799,093	1.13 (0.55–2.31)	20	1,040,872	1.55 (0.65–3.68)	23	1,040,872	1.63 (0.64–4.17)
Q3 (24.5–33.2)	43	761,407	1.38 (0.68–2.78)	20	954,814	1.18 (0.46–3.02)	37	954,814	1.54 (0.60–3.95)
Q4 (33.2–244.6)	39	705,551	0.98 (0.47–2.0)	–	–	–	–	–	–
<i>Sensitivity analysis</i>									
<i>Excluding first 2 years of follow-up</i>									
Quartiles (µg/day)									
Estimated acrylamide intake (µg/day)									
Q1 (0–15.6)	27	1,297,226	reference	19	1,297,226	reference	7	1,297,226	reference
Q2 (15.7–23.3)	65	1,296,721	1.69 (1.05–2.73)	36	1,296,721	1.60 (0.88–2.91)	24	1,296,721	2.16 (0.86–8.46)
Q3 (23.4–30.7)	84	1,321,604	1.46 (0.89–2.39)	39	1,321,604	1.30 (0.69–2.44)	38	1,321,604	1.97 (0.78–4.96)
Q4 (34.2–261.4)	111	1,338,682	1.30 (0.76–2.21)	48	1,338,682	1.22 (0.61–2.42)	54	1,338,682	1.68 (0.64–4.42)
Energy-adjusted acrylamide intake (µg/day)									
Q1 (0–17.7)	40	1,304,405	reference	25	1,304,405	reference	12	1,304,405	reference
Q2 (17.8–24.4)	61	1,287,225	1.31 (0.85–2.04)	38	1,287,225	1.56 (0.88–2.74)	20	1,287,225	1.26 (0.57–2.80)
Q3 (24.5–33.2)	78	1,325,145	1.13 (0.73–1.77)	31	1,325,145	0.92 (0.50–1.70)	42	1,325,145	1.53 (0.72–3.26)
Q4 (33.2–244.6)	108	1,337,459	1.10 (0.70–1.72)	48	1,337,459	1.11 (0.60–2.04)	49	1,337,459	1.14 (0.53–2.46)

^a PY = person-years^b Adjusted for sex, total energy (kcal/d), total fruits (g/d), cigarette smoking status, number of cigarettes (c/d), time since quitting smoking (y), processed meat (g/d) and alcohol (g/d) and stratified by age and country. BMI was not included because it is a risk factor for EAC and a possible protective factor for ESCC^c Adjusted for sex, total energy (kcal/d), total fruits (g/d), cigarette smoking status, number of cigarettes (c/d), time since quitting smoking (y), processed meat (g/d), BMI (kg/m²) and alcohol (g/d) and stratified by age and country^d Adjusted for sex, total energy (kcal/d), total fruits (g/d), cigarette smoking status, number of cigarettes (c/d), time since quitting smoking (y), processed meat (g/d) and BMI (kg/m²) and stratified by age and country. Alcohol was not included because it is not considered a risk factor for EAC^e For ESCC and EAC, tertiles of acrylamide were analyzed (T1: 0–18.1, T2: 18.2–29.75, T3: 29.76–261.4)^f For ESCC and EAC, tertiles of energy-adjusted acrylamide were analyzed (T1: 0–20.03, T2: 20.04–29.63, T3: 29.64–244.6)

composition tables and used different cut-points for acrylamide intake in the analyses (although results in EPIC were unchanged when NLCS cut-points were used). The NLCS, the Italian, and the Swedish case-control studies estimated acrylamide intakes from country-specific acrylamide databases [4–6], while the EPIC study was based on the IRMM [3, 8]; thus, direct comparison of the results of these studies with EPIC should be made with caution.

Acrylamide was discovered in food in 2002 [2], and it has been shown that levels can vary in a single item by a factor of 100 or more depending on factors such as the cooking method and brand [2, 3]. We acknowledge some uncertainty in how well dietary acrylamide intake is captured by EPIC DQs (for example, information on extent of cooking which could influence acrylamide levels in foods were not accounted for). A recent publication from our group showed a low correlation between DQs and a single 24HDR (0.17) [13], but a single 24HDR may not be adequate to estimate daily acrylamide intake levels. The major strength of our study is that it is the largest prospective study of dietary acrylamide intake and EC to date.

In conclusion, results from the EPIC cohort suggest that estimated acrylamide intake is not linearly associated with an increased risk of developing EC. Although statistically significant elevated risks were observed in the middle quartiles for total EC, no statistically significant elevated risks were observed for the fourth quartile, neither for EAC or ESCC when analyzed separately nor when the estimation of dietary acrylamide intake was corrected using the residual method. Results from other large cohorts and consortia, and results from biomarker studies, might add to the evidence provided by this analysis, suggesting that acrylamide is not an important risk factor for EC.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. IARC (1994) IARC working group on the evaluation of carcinogenic risks to humans: some industrial chemicals. Lyon. IARC Monogr Eval Carcinog Risks Hum 60:1–560
2. Pelucchi C, La Vecchia C, Bosetti C, Boyle P, Boffetta P (2011) Exposure to acrylamide and human cancer—a review and meta-analysis of epidemiologic studies. *Ann Oncol* 22(7):1487–1499
3. Freisling H, Moskal A, Ferrari P et al (2013) Dietary acrylamide intake of adults in the European Prospective Investigation into cancer and nutrition differs greatly according to geographical region. *Eur J Nutr* 52(4):1369–1380
4. Lin Y, Lagergren J, Lu Y (2011) Dietary acrylamide intake and risk of esophageal cancer in a population-based case-control study in Sweden. *Int J Cancer* 128(3):676–681
5. Pelucchi C, Galeone C, Levi F et al (2006) Dietary acrylamide and human cancer. *Int J Cancer* 118(2):467–471
6. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2008) Dietary acrylamide intake is not associated with gastrointestinal cancer risk. *J Nutr* 138(11):2229–2236
7. Riboli E, Hunt KJ, Slimani N et al (2002) European prospective investigation into cancer and nutrition (EPIC): study populations and data collection. *Public Health Nutr* 5(6B):1113–1124
8. Obon-Santacana M, Slimani N, Lujan-Barroso L et al (2013) Dietary intake of acrylamide and pancreatic cancer risk in the European prospective investigation into cancer and nutrition (EPIC) cohort. *Ann Oncol* 24(10):2645–2651
9. Steffen A, Schulze MB, Pischon T et al (2009) Anthropometry and esophageal cancer risk in the European prospective investigation into cancer and nutrition. *Cancer Epidemiol Biomarkers Prev* 18(7):2079–2089
10. Holmes RS, Vaughan TL (2007) Epidemiology and pathogenesis of esophageal cancer. *Semin Radiat Oncol* 17(1):2–9
11. Schoenfeld D (1982) Partial residuals for the proportional hazards regression model. *Biometrika* 69(1):239–241
12. Heinzl H, Kaider A (1997) Gaining more flexibility in Cox proportional hazards regression models with cubic spline functions. *Comput Methods Programs Biomed* 54(3):201–208
13. Ferrari P, Freisling H, Duell EJ et al (2013) Challenges in estimating the validity of dietary acrylamide measurements. *Eur J Nutr* 52(5):1503–1512
14. Willett W (1998) *Nutritional Epidemiology*. Second ed
15. Blot WJ, McLaughlin JK, Fraumeni JF Jr (2006) Esophageal cancer. In: Schottenfeld D, Fraumeni JF Jr (eds) *Cancer epidemiology and prevention*, 3rd edn. Oxford, New York, pp 697–706
16. Smith M, Zhou M, Whitlock G et al (2008) Esophageal cancer and body mass index: results from a prospective study of 220,000 men in China and a meta-analysis of published studies. *Int J Cancer* 122(7):1604–1610
17. Koppert LB, Wijnhoven BP, van Dekken H, Tilanus HW, Dinjens WN (2005) The molecular biology of esophageal adenocarcinoma. *J Surg Oncol* 92(3):169–190
18. Jakszyn P, Lujan-Barroso L, Agudo A et al (2013) Meat and heme iron intake and esophageal adenocarcinoma in the European prospective investigation into cancer and nutrition study. *Int J Cancer* 133(11):2744–2750

19. Salehi M, Moradi-Lakeh M, Salehi MH, Nojomi M, Kolaheidoz F (2013) Meat, fish, and esophageal cancer risk: a systematic review and dose-response meta-analysis. *Nutr Rev* 71(5):257–267
20. Steffen A, Bergmann MM, Sanchez MJ et al (2012) Meat and heme iron intake and risk of squamous cell carcinoma of the upper aero-digestive tract in the European prospective investigation into cancer and nutrition (EPIC). *Cancer Epidemiol Biomarkers Prev* 21(12):2138–2148
21. Boeing H, Dietrich T, Hoffmann K et al (2006) Intake of fruits and vegetables and risk of cancer of the upper aero-digestive tract: the prospective EPIC-study. *Cancer Causes Control* 17(7):957–969
22. Gonzalez CA, Pera G, Agudo A et al (2006) Fruit and vegetable intake and the risk of stomach and oesophagus adenocarcinoma in the European prospective investigation into cancer and nutrition (EPIC-EURGAST). *Int J Cancer* 118(10):2559–2566

Null Results in Brief

Cancer
Epidemiology,
Biomarkers
& Prevention

Dietary Acrylamide Is Not Associated with Renal Cell Cancer Risk in the CPS-II Nutrition Cohort

Marjorie L. McCullough, Rebecca A. Hodge, Caroline Y. Um, and Susan M. Gapstur



Abstract

Background: Acrylamide, an industrial chemical and probable human carcinogen, can be formed in primarily carbohydrate-containing foods during high-heat cooking or processing. Most epidemiologic studies show no associations of dietary acrylamide intake with most cancer outcomes, but limited prospective evidence suggests a positive association with renal cell carcinoma (RCC).

Methods: In 1999, 102,154 men and women from the Cancer Prevention Study-II Nutrition Cohort completed a questionnaire on diet, lifestyle, and cancer risk factors and were followed through June 30, 2013. Cox proportional hazards regression was used to estimate the HR and 95% confidence interval (CI) for the association

between estimated dietary acrylamide intake and risk of RCC.

Results: After 1,137,441 person-years of follow-up, 412 cases of invasive RCC occurred. In multivariable-adjusted models, there was no association between acrylamide intake and risk of RCC (HR = 1.09; 95% CI, 0.82–1.43) for the highest versus lowest quartile of intake. Associations were not modified by sex or smoking history.

Conclusions: We found no associations between dietary acrylamide exposure and risk of invasive RCC.

Impact: The findings from this large, prospective analysis do not support a positive association between higher dietary acrylamide intake and RCC risk.

Introduction

Acrylamide, an industrial chemical, is classified as a probable human carcinogen (1). In daily life, tobacco smoke is a major source of exposure (2). However, in 2002, Swedish scientists discovered that acrylamide could be formed in (mostly) carbohydrate-containing foods during high-heat cooking (3). This raised concerns that humans could be exposed to large doses of this potentially harmful chemical through diet (4).

A meta-analysis of 32 epidemiologic studies (5) indicated that dietary acrylamide is not related to risk of most common types of cancer. However, for renal cell cancer (RCC), the relative risk (RR) was 1.20 [95% confidence interval (CI), 1.00–1.45; Q4 vs. Q1]. When limited to the two published prospective studies (6, 7), the combined RR was 1.48 (95% CI, 1.09–2.00) for high versus low exposure (5).

To contribute to the limited evidence base, we examined the association between estimated dietary acrylamide and RCC risk, overall and by sex and smoking status, in the prospective Cancer Prevention Study (CPS)-II Nutrition Cohort.

Materials and Methods

The CPS-II Nutrition Cohort was established by the American Cancer Society in 1992 (8). In 1999, 151,337 participants returned a 152-item–modified Harvard food frequency questionnaire (FFQ), for which dietary acrylamide content was derived; thus, 1999 served as baseline for this analysis. We excluded those who were lost to follow-up ($n = 5,742$), reported kidney cancer ($n = 457$) or another cancer ($n = 27,405$) before 1999, had unverified self-reported kidney cancer during the first follow-up interval ($n = 9$), were diagnosed >6 months after self-report ($n = 2$), or had poor FFQ information ($n = 15,568$). This left 102,154 participants for analysis. The CPS-II Nutrition Cohort is approved by the Emory University Institutional Review Board.

The 1999 modified Harvard FFQ was previously validated for acrylamide assessment using hemoglobin adducts of acrylamide and its metabolite, glycidamide (9). Participants were asked how often they consumed each item listed on the FFQ, on average, over the past year. Frequency categories ranged from never/rarely to 4+ times/day for food and 6+ times/day for beverages. Acrylamide intake was calculated as described previously (9), energy-adjusted using the residual method, and categorized into sex-specific quartiles.

RCC cases were identified through self-reports of cancer on biennial surveys ($n = 302$) that were verified by medical records ($n = 215$) or state cancer registry linkage ($n = 87$). An additional 110 cases were identified from death certificates listing kidney cancer as the primary cause of death (ICD C64), ascertained through biennial computerized linkage of the entire cohort with the National Death Index; 61 of these cases were subsequently verified by state cancer registry linkage. In total, 412 men and women with primary, invasive RCC were included in this analysis.

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

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Table 1. Participant characteristics according to sex-specific quartiles of dietary acrylamide intake

	Q1 (n = 25,539)	Q2 (n = 25,538)	Q3 (n = 25,539)	Q4 (n = 25,538)
Mean (SD) acrylamide (µg/d)	13.4 (3.0)	18.7 (2.7)	23.2 (3.4)	33.0 (9.9)
Men	15.3 (2.8)	21.5 (1.4)	26.7 (1.7)	39.1 (10.7)
Women	12.0 (2.2)	16.6 (1.0)	20.5 (1.3)	28.3 (6.2)
Sociodemographic characteristics				
Women (%)	56.8	56.8	56.8	56.8
Age (y) ^a	69.4 (6.2)	69.5 (6.1)	69.3 (6.0)	68.8 (6.1)
Physical activity (MET hrs/wk) ^{a,b}	16.7 (16.4)	16.7 (15.5)	16.3 (15.4)	14.9 (14.5)
Race (%)				
White	96.6	97.7	98.2	98.3
Non-white	3.4	2.3	1.8	1.7
Body mass index (kg/m ² ; %)				
<18.5	1.4	1.4	1.1	1.2
18.5–<25	42.8	42.5	41.7	38.9
25–<30	38.2	38.8	39.6	40.3
≥30	15.6	15.5	15.9	17.7
Missing	2.0	1.8	1.7	1.9
Smoking status ^c (%)				
Never smoker	49.4	47.4	45.9	43.2
Former, quit ≥ 30 y	22.5	22.9	23.2	21.9
Former, quit 20–<30 y	9.8	10.6	10.7	11.2
Former, quit 10–<20 y	8.5	9.4	9.1	10.2
Former, quit <10 y	5.9	5.8	6.3	7.4
Current smoker	3.9	3.8	4.7	6.1
Highest education level (%)				
High school or less	26.7	28.3	30.7	35.6
Vocational/some college	29.1	29.3	28.7	29.6
College graduate or higher	44.2	42.4	40.6	34.8
Alcohol consumption (%)				
Nondrinker	25.6	23.7	24.0	27.7
>0–0.5 drinks/d	40.6	43.6	45.7	47.5
>0.5–1 drink/d	12.5	13.5	13.9	12.2
>1–2 drinks/d	9.9	10.4	9.6	7.6
>2 drinks/d	11.3	8.8	6.8	4.9
History of diabetes (yes; %)	10.4	11.3	12.2	15.1
History of hypertension (yes; %)	59.6	61.3	61.1	60.2
History of kidney stones (yes; %)	5.2	5.2	5.2	5.2
History of kidney disease (yes; %)	1.4	1.5	1.5	1.4
Family history of kidney cancer (yes; %)	0.8	0.8	0.8	0.9
Diet^a				
Energy (kcal)	1,796 (587)	1,806 (559)	1,735 (520)	1,598 (515)
Dietary fiber (g)	17.3 (5.0)	18.4 (4.9)	18.9 (5.0)	19.0 (5.3)
Coffee (regular and decaf; svg/d)	0.9 (1.0)	1.4 (1.2)	1.8 (1.4)	2.3 (1.7)
Fruits and vegetables (svg/d)	5.1 (2.5)	5.0 (2.4)	4.8 (2.4)	4.2 (2.2)
Red and processed meat (svg/d)	1.0 (0.8)	1.0 (0.7)	0.9 (0.7)	0.9 (0.7)
Calories from fat (%)	30.8 (7.5)	30.2 (6.6)	30.2 (6.4)	30.7 (6.4)
Calories from protein (%)	15.9 (3.3)	15.9 (2.9)	15.8 (2.7)	15.6 (2.7)
Calories from carbohydrates (%)	52.2 (9.5)	53.4 (8.1)	53.8 (7.9)	53.9 (7.9)

Abbreviations: d, day; g, grams; hrs, hours; kcal, kilocalories; kg, kilograms; svg, servings; wk, week; y, years; µg, micrograms.

^aValues are mean (SD).^bSome participants are missing physical activity information (n = 1,803; 1.8%).^cParticipants missing smoking status (1.0%) were added to the largest category of former smokers (former, quit ≥30 y).

Follow-up began with return of the 1999 survey and continued until date of RCC diagnosis or death, censoring due to loss to follow-up, other cause of death, or June 30, 2013, whichever came first. Cox proportional hazards regression, stratifying on 1-year age intervals and adjusting for sex, estimated the HR and corresponding 95% CI. Multivariable-adjusted models additionally adjusted for body mass index (kg/m²), history of hypertension, history of diabetes, smoking status, physical activity, alcohol consumption, and total energy intake at baseline.

P_{trend} was calculated using a continuous variable created from medians within sex-specific acrylamide quartiles. We examined

heterogeneity in associations by smoking status (never/ever) and sex. Likelihood ratio tests were used to test violations of the Cox proportional hazards assumption and heterogeneity of associations. Statistical analyses used SAS version 9.4 (SAS Institute). *P* values were two-sided and considered statistically significant if <0.05.

Results

At baseline, mean energy-adjusted (SD) acrylamide intakes were 19.4 (6.9) µg/day in women, and 25.7 (10.4) µg/day in men. The top three contributors to acrylamide exposure were

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Table 2. Age- and multivariable-adjusted HRs and 95% CI for incident renal cell cancer by dietary acrylamide intake in 1999, overall, and by sex in the CPS-II Nutrition Cohort (1999–2013)

	Q1 (ref) ^a	Q2	Q3	Q4	P _{trend}	Acrylamide (10 µg/d)
Men and women combined						
Incident cases (n)	96	106	98	112	—	—
Person-years	280,402	285,429	286,363	285,247	—	—
Age- and sex-adjusted model	1.00	1.08 (0.82–1.42)	1.00 (0.75–1.32)	1.16 (0.88–1.52)	0.45	1.06 (0.96–1.17)
Multivariable model ^b	1.00	1.06 (0.81–1.40)	0.96 (0.73–1.28)	1.09 (0.82–1.43)	0.82	1.03 (0.93–1.14)
Men						
Incident cases (n)	57	71	59	62	—	—
Person-years	115,679	117,811	118,406	118,266	—	—
Age-adjusted model	1.00	1.21 (0.85–1.72)	1.01 (0.70–1.45)	1.08 (0.75–1.54)	0.96	1.03 (0.92–1.16)
Multivariable model ^b	1.00	1.20 (0.85–1.70)	0.96 (0.66–1.38)	0.97 (0.68–1.41) ^c	0.60	1.00 (0.89–1.13)
Women						
Incident cases (n)	39	35	39	50	—	—
Person-years	164,723	167,617	167,957	166,981	—	—
Age-adjusted model	1.00	0.88 (0.56–1.39)	0.98 (0.63–1.53)	1.28 (0.84–1.95)	0.15	1.16 (0.94–1.43)
Multivariable model ^b	1.00	0.87 (0.55–1.37)	0.97 (0.62–1.51)	1.26 (0.83–1.93) ^c	0.17	1.15 (0.93–1.42)

^aQuartiles were sex-specific. Cut-off points for men and women, respectively, were as follows (µg/d): Q1 (<19.0; <14.8); Q2 (<23.9; <18.4); Q3 (<29.9; <22.9); and Q4 (≥29.9; ≥22.9).

^bAdjusted for age, sex (except in sex-specific models), body mass index category (<18.5 kg/m²; 18.5–<25 kg/m²; 25–<30 kg/m²; ≥30 kg/m²; and missing), smoking status [never smoker; former smoker (quit ≥30 y/missing); former (quit 20–<30 y); former (quit 10–<20 y); former (quit <10 y); and current smoker], diabetes (yes/no), hypertension (yes/no), physical activity (<8.75 MET hrs/wk; 8.75–<17.5 MET hrs/wk; ≥17.5 MET hrs/wk; and missing), alcohol consumption (0 drinks/d; >0–0.5 drinks/d; >0.5–1 drinks/d; >1–2 drinks/d; and >2 drinks/d), and total energy intake (sex-specific quartiles).

^cP_{heterogeneity} by sex = 0.19.

french fries (23% of intake), coffee (15%), and bread (10%). Those in the higher intake quartiles tended to be less educated, heavier, current or former smokers, have diabetes, and to consume less alcohol (Table 1).

Acrylamide intake was not associated with RCC among men, women, or both combined after adjusting for age (and sex) or in multivariable analyses (Table 2). These associations were not modified by smoking history ($P_{\text{heterogeneity}} = 0.62$; Supplementary Table S1).

Discussion

In this prospective analysis of 102,154 U.S. adults, estimated dietary acrylamide intake was not associated with invasive RCC risk, in age- and sex-adjusted models or in models controlling for other RCC risk factors. Likewise, associations did not differ among strata defined by smoking status or sex.

We undertook this analysis because of previously reported positive associations of dietary acrylamide and RCC risk in two prospective cohort studies (6, 7). This analysis included more ($n = 412$) cases than the previous studies (339; ref. 6) and (184; ref. 7), with a similar range of estimated exposure to one (6) study and a slightly lower range than the other (7). In conclusion, we found no evidence that greater dietary acrylamide intake was associated with risk of RCC.

References

1. International Agency for Research on Cancer/WHO. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Some industrial chemicals, volume 60. Lyon, France: International Agency for Research on Cancer; 1994. Available from: <https://monographs.iarc.fr/wp-content/uploads/2018/06/mono60.pdf>.
2. Vesper HW, Bernert JT, Ospina M, Meyers T, Ingham L, Smith A, et al. Assessment of the relation between biomarkers for smoking and biomarkers for acrylamide exposure in humans. *Cancer Epidemiol Biomarkers Prev* 2007;16:2471–8.
3. Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem* 2002;50:4998–5006.
4. Kapp C. WHO urges more research into acrylamide in food. *Lancet* 2002;360:64.
5. Pelucchi C, Bosetti C, Galeone C, La Vecchia C. Dietary acrylamide and cancer risk: an updated meta-analysis. *Int J Cancer* 2015;136:2912–22.
6. Hogervorst JC, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. Dietary acrylamide intake and the risk of renal

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The views expressed here are those of the authors and do not necessarily represent the American Cancer Society or the American Cancer Society – Cancer Action Network.

Authors' Contributions

Conception and design: M.L. McCullough
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.M. Gapstur
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.L. McCullough, R.A. Hodge
Writing, review, and/or revision of the manuscript: M.L. McCullough, C.Y. Um, S.M. Gapstur

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- cell, bladder, and prostate cancer. *Am J Clin Nutr* 2008;87:1428–38.
7. Hirvonen T, Kontto J, Jestoi M, Valsta L, Peltonen K, Pietinen P, et al. Dietary acrylamide intake and the risk of cancer among Finnish male smokers. *Cancer Causes Control* 2010;21:2223–9.
 8. Calle EE, Rodriguez C, Jacobs EJ, Almon ML, Chao A, McCullough ML, et al. The American Cancer Society Cancer Prevention Study II Nutrition Cohort – rationale, study design, and baseline characteristics. *Cancer* 2002;94:2490–501.
 9. Wilson KM, Vesper HW, Tocco P, Sampson L, Rosen J, Hellenas KE, et al. Validation of a food frequency questionnaire measurement of dietary acrylamide intake using hemoglobin adducts of acrylamide and glycidamide. *Cancer Causes Control* 2009;20:269–78.

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Dietary Acrylamide Is Not Associated with Renal Cell Cancer Risk in the CPS-II Nutrition Cohort

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Supplementary Table 1: Age- and multivariable-adjusted rate ratios (RR) and 95% confidence intervals (CI) for incident renal cell cancer by dietary acrylamide intake in 1999 in the CPS-II Nutrition Cohort (1999-2013), stratified by smoking

	Q1 (Ref) ^a	Q2	Q3	Q4	<i>p</i> -trend
Never Smokers (n=47,503)					
Incident cases (n)	39	42	43	37	
Person-years	142,842	139,777	135,267	127,104	
Age and sex adjusted model	1.00	1.11 (0.72, 1.72)	1.18 (0.76, 1.81)	1.10 (0.70, 1.72)	0.74
Multivariable model ^b	1.00	1.09 (0.71, 1.69)	1.15 (0.75, 1.78)	1.07 (0.68, 1.69) ^c	0.83
Ever Smokers (n=54,651)					
Incident cases (n)	57	64	55	75	
Person-years	137,560	145,651	151,096	158,142	
Age and sex-adjusted model	1.00	1.05 (0.73, 1.50)	0.87 (0.60, 1.26)	1.15 (0.81, 1.62)	0.63
Multivariable model ^b	1.00	1.04 (0.73, 1.49)	0.83 (0.58, 1.21)	1.06 (0.74, 1.50) ^c	0.95

^a Quartiles were sex-specific. Cut points for men and women, respectively, were as follows (µg/d): Q1 (<19.0; <14.8); Q2 (<23.9; <18.4); Q3 (<29.9; <22.9); Q4 (≥ 29.9; ≥ 22.9)

^b Adjusted for age, sex, BMI category (<18.5 kg/m²; 18.5-<25; 25-<30; ≥30; missing), diabetes (yes/no), hypertension (yes/no), physical activity (<8.75 MET hrs/wk; 8.75-<17.5; ≥17.5; missing), alcohol consumption in 1999 (0 drinks/day; >0-0.5 drinks/day; >0.5-1 drinks/day; >1-2 drinks/day; >2 drinks per day), and total energy intake (sex-specific quartiles)

^c *p*-value, heterogeneity by smoking=0.62

20. Miller PE, Vasey JJ, Short PF et al. Dietary supplement use in adult cancer survivors. *Oncol Nurs Forum* 2009; 36: 61–68.
21. Davidson R, Geoghegan L, McLaughlin L et al. Psychological characteristics of cancer patients who use complementary therapies. *Psychooncology* 2005; 14: 187–195.
22. Hann D, Baker F, Denniston M et al Long-term breast cancer survivors' use of complementary therapies: perceived impact on recovery and prevention of recurrence. *Integr. Cancer Ther* 2005; 4.1: 14–20.
23. Verhoef MJ, Trojan L, Armitage GD et al. Complementary therapies for cancer patients: assessing information use and needs. *Chronic Dis Can* 2009; 29: 80–88.
24. Frenkel M, Ben-Arye E, Cohen L. Communication in cancer care: discussing complementary and alternative medicine. *Integr Cancer Ther* 2010; 9: 177–185.
25. Boddy K, Ernst E. Review of reliable information sources related to integrative oncology. *Hematol Oncol Clin North Am* 2008; 22: 619–630, vii.

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Dietary intake of acrylamide and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort

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Background: In 1994, acrylamide (AA) was classified as a probable human carcinogen by the International Agency for Research on Cancer. In 2002, AA was discovered at relatively high concentrations in some starchy, plant-based foods cooked at high temperatures.

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Patients and methods: A prospective analysis was conducted to evaluate the association between the dietary intake of AA and ductal adenocarcinoma of the exocrine pancreatic cancer (PC) risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort using Cox regression modeling. EPIC includes >500 000 men and women aged 35–75 at enrollment from 10 European countries. AA intake was estimated for each participant by combining questionnaire-based food consumption data with a harmonized AA database derived from the EU monitoring database of AA levels in foods, and evaluated in quintiles and continuously.

Results: After a mean follow-up of 11 years, 865 first incident adenocarcinomas of the exocrine pancreas were observed and included in the present analysis. At baseline, the mean dietary AA intake in EPIC was 26.22 µg/day. No overall association was found between continuous or quintiles of dietary AA intake and PC risk in EPIC (HR:0.95, 95% CI:0.89–1.01 per 10 µg/day). There was no effect measure modification by smoking status, sex, diabetes, alcohol intake or geographic region. However, there was an inverse association (HR: 0.73, 95% CI: 0.61–0.88 per 10 µg/day) between AA intake and PC risk in obese persons as defined using the body mass index (BMI, ≥ 30 kg/m²), but not when body fatness was defined using waist and hip circumference or their ratio.

Conclusions: Dietary intake of AA was not associated with an increased risk of PC in the EPIC cohort.

Key words: acrylamide, cohort, nutrition, pancreatic cancer

introduction

Pancreatic cancer is the fourth and fifth most common cause of cancer mortality in both sexes combined in the U.S. and European Union, respectively; and 5-year survival rates are among the lowest (<5%) for any cancer [1, 2]. Over 95% are ductal adenocarcinomas of the exocrine pancreatic cancer (PC), here referred to as pancreatic cancer (PC) [3]. At least 20% of the disease is attributable to tobacco smoking [4]. Other PC risk factors include long-standing diabetes, history of pancreatitis, overweight and obesity, non-O blood group, *Helicobacter pylori* infection and possibly heavy alcohol consumption [5].

Acrylamide (AA), classified by the International Agency for Research on Cancer ‘probably carcinogenic’ to humans in 1994 [6], was discovered as a preparation by-product in some foods in 2002 [7]. Tobacco is also an important source of AA exposure, and smokers have mean AA hemoglobin adducts levels at least three to four times higher than nonsmokers [8, 9].

AA in certain cooked foods is formed primarily through the Maillard reaction between reducing sugars and asparagines during baking, frying, grilling and other high temperature (>120°C) food preparations [7]. The top AA sources differ across European countries due to differences in dietary habits related to cooking practices as well as ingredients [10]. As a consequence, dietary intake of AA for individuals in populations such as the European Prospective Investigation into Cancer and Nutrition (EPIC) differs by about threefold across 10 countries in the cohort [11].

AA is metabolized in the body to a chemically reactive epoxide, glycidamide (GA), in a reaction catalyzed by the cytochrome P450 enzyme complex CYP2E1. GA is a known genotoxin and animal carcinogen [12]. Studies in rats and mice have shown that an oral administration of AA increases different types of hormonal and non-hormonal tumor rates [12].

Three epidemiologic studies have assessed the association between dietary AA exposure and PC risk: two prospective cohort studies, The Netherlands Cohort Study (NLCS) [13] and the Alfa-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study conducted in Finland [14], and a case-control study conducted from 1991 to 2008 in Northern Italy [15]. Overall,

these studies did not observe any association, but each study evaluated <349 PC cases, and utilized different designs and had different ranges of AA intake.

The present study evaluated the association between the questionnaire-based dietary intake of AA and the risk of PC, using data from 477 308 participants, including 865 PC cases in the EPIC cohort.

methods

study population

EPIC is a multicenter prospective cohort study that includes 521 330 participants recruited between 1992 and 1998 from 23 research centers in 10 European countries (listed in Table 1). The methods have been reported in detail by Riboli et al. [16].

Of the 521 330 participants, a total of 44 022 were excluded because they were diagnosed with cancer before recruitment ($n = 23\,785$), had incomplete follow-up data ($n = 4380$), no lifestyle or dietary information or information on dietary intake of AA at baseline ($n = 6257$), or had an extreme ratio of energy intake to energy required ($n = 9600$), resulting in 477 308 participants for this analysis.

identification of pancreatic cancer cases

Cancer incidence information was assessed through population cancer registries or through a combination of three methods that included: health insurance records, cancer and pathology registries, and active follow-up [16]. Follow-up time was defined as the interval that began at the date of recruitment and ended at the date of last complete follow-up or PC diagnosis or death, whichever occurred first. Forty-five cases were censored because they were neuroendocrine pancreatic tumors ($n = 42$), a benign tumor ($n = 1$), a carcinoma *in situ* ($n = 1$), or a tumor with uncertain primary origin ($n = 1$). After a mean follow-up of 11 years, 865 first incident PCs were available for analysis and were classified corresponding to the International Classification of Diseases 10th revision as C25 (C25.0–C25.3 and C25.7–C25.9).

dietary and AA intake assessment

Food consumption and baseline alcohol intake in EPIC was assessed at cohort enrollment by country-specific and validated food intake questionnaires. Information on AA levels in food was obtained from the European Community Institute for Reference Materials and Measurements

Table 1. Dietary intake of acrylamide (AA), and pancreatic cancer (PC) cases in the EPIC cohort by country and sex

Country	Cohort sample	Person-years	Pancreatic adenocarcinoma cases	AA intake (µg/day)	
				Mean ± SD	Median
Men					
Denmark	26 294	284 721	118	43.23 ± 13.77	42.27
France ^a	—	—	—	—	—
Germany	21 172	208 509	64	30.51 ± 13.54	28.03
Greece	10 807	99 108	20	24.00 ± 13.19	21.60
Italy	14 029	158 917	27	11.45 ± 6.65	10.04
Norway ^a	—	—	—	—	—
Spain	15 148	182 965	28	27.76 ± 16.04	24.88
Sweden	22 308	289 607	73	29.17 ± 12.77	26.96
The Netherlands	9 639	115 570	15	38.41 ± 16.26	36.13
UK	22 852	252 096	51	39.10 ± 17.79	36.51
Total	142 249	1 591 493	396	31.90 ± 16.91	29.69
Women					
Denmark	28 722	316 745	71	35.51 ± 11.68	34.48
France	67 382	699 332	46	20.36 ± 8.78	19.14
Germany	27 411	272 105	41	24.45 ± 11.15	22.38
Greece	15 225	148 604	16	19.03 ± 9.01	17.51
Italy	30 512	341 489	41	10.86 ± 6.12	9.64
Norway	35 169	342 279	20	17.92 ± 6.48	17.37
Spain	24 854	299 617	31	20.50 ± 12.14	18.25
Sweden	26 375	349 308	79	22.36 ± 9.71	20.63
The Netherlands	26 866	315 683	52	31.02 ± 13.60	29.06
UK	52 543	586 301	72	32.94 ± 15.22	30.57
Total	335 059	3 671 462	469	23.81 ± 13.07	21.26
TOTAL	477 308	5 262 954	865	26.22 ± 14.79	23.29

EPIC, European Prospective Investigation into Cancer and Nutrition; SD, standard deviation.

^aCohorts from France and Norway were in women only.

(IRMM) database. Methods were based on either liquid or gas chromatography coupled to mass spectrometry. The IRMM database includes AA levels in foods mainly from Austria, Germany, Greece, Ireland, The Netherlands, the UK, and from the food industry.

An inventory of all food items occurring in the IRMM database and the additional sources for the 10 countries included in the present study was made and classified according to EPIC-Soft [17]. The dietary questionnaire (DQ) foods and, when available, their specific description (e.g. 'baked potatoes') were matched with the corresponding foods in the AA database. In general, DQ consumption factors on the proportion of the cooking method for a given food were derived from the distribution of specific foods (e.g. boiled, fried, or roasted potatoes) using national consumption data [11]. If an exact match was not possible, the item was paired to a mean of all foods of the food group in the AA database. Relevant information on food preparation was available for potatoes (except in Italy), bread, and breaded meats. The main determinants of dietary intake of AA in the EPIC cohort based on 24-h dietary recall (24HDR) were bread, crispbread, rusks, coffee, potatoes, cakes, biscuits, and cookies [11].

lifestyle information and covariates assessment

Information on lifestyle factors was collected at cohort enrollment using lifestyle questionnaires [16]. Baseline height, weight, and waist or hip circumference were measured by trained personnel according to the standardized procedures [18], except for the majority of the French, Norwegian, and Oxford cohorts, where height and weight were self-

reported. Umeå, Norway, and France did not collect data on the waist or hip circumference [16].

statistical analysis

Two continuous variables for dietary intake of AA were created: average daily AA intake in micrograms per day (here referred to as 'AA intake'), and intake per 10 µg increment of AA (10 µg/day). AA intake was also categorized into quintiles based on the distribution in the entire EPIC cohort.

HRs for AA intake and PC risk were estimated using proportional hazards models (Cox regression) with age as the time scale and stratification by age at recruitment (in 1-year categories), and study center. The following variables were investigated as known risk factors or potential confounders in these analyses: sex, body mass index (BMI, kg/m²), smoking status (never smokers, current pipe or cigar or occasional smokers, current cigarette smokers: 1–15, 16–25, or ≥26 cigarettes/day, former cigarette smokers who quit >20 years, 11–20 years, or ≤10 years before recruitment), history of diabetes (no, yes), alcohol intake (nondrinkers, drinkers of 0–6, >6–12, >12–24, >24–60 g/day, female drinkers >60 g/day, male drinkers >60–96 g/day, and male drinkers >96 g/day), education level (none, primary, technical/professional, secondary, higher education), physical activity using the Cambridge index [19], total energy (per 1000 kcal/day), total fat (g/day), total fiber (g/day), vegetable (g/day), fruits, nuts and seeds (g/day), red meat (g/day), and processed meat consumption (g/day). Covariates of sex, BMI, smoking status, history of diabetes, and alcohol intake remained in models because they changed HR estimates ≥10% or were known risk factors for

PC. Total energy intake was included in all statistical models. Total carbohydrate intake and coffee intake were investigated for association with total AA intake; however, since these are sources of AA exposure in the diet, they were not included in multivariable models.

Analyses were stratified by smoking status in order to isolate the potential effect of dietary AA exposure from smoking-related AA exposure on PC risk. Models were also stratified by sex, diabetes status, baseline alcohol intake (nondrinkers, drinkers <24 g/day, drinkers 24–<60 g/day, and heavy drinkers ≥60 g/day), and BMI [underweight/normal weight (<25 kg/m²), overweight (25–<30 kg/m²), and obese (≥30 kg/m²)]. Stratified analyses by alcohol intake and BMI were carried out since these factors may affect the activity of CYP2E1 [20]. For analyses by geographic region, countries were classified as north (France, the UK, The Netherlands, Germany, Sweden, Denmark, and Norway) and south (Italy, Spain, and Greece) and by AA intake level: high ≥24 μg/day and low <24 μg/day (Table 1). Germany and Sweden had intermediate AA intake levels, so models were run with and without these countries.

We evaluated four variables as measures of overweight and obesity: BMI, waist circumference, hip circumference, and waist-to-hip ratio (WHR).

The median value for each AA quintile was estimated, and these values were used in order to evaluate dose-response trends. Interaction was evaluated using the likelihood ratio test. The proportional hazards assumption was evaluated using Schoenfeld residuals [21].

The Goldberg criteria, a measure for identifying under-reporters of energy intake [22], was used with the aim of assessing whether under-estimation of self-reported food intake and, as a consequence, energy intake could influence the results. This variable was used in two ways: added as a covariate in Cox models; and to restrict the analysis to those participants who had plausible energy intakes.

To account for the possible influence of preclinical disease on dietary habits and estimates of AA intake, a sensitivity analysis was carried out by excluding the cases diagnosed during the first 2 years of follow-up. Additional sensitivity analyses restricting the cases to microscopically confirmed PC ($n = 608$ or 70.3%, using any combination of cytology or hematology, histology/cytology of metastasis, histology/cytology of primary tumor, and examination at autopsy) were also carried out.

results

Patterns of dietary intake of AA in the EPIC cohort by country and sex show that the country with the highest mean and median for AA intake in men and women was Denmark, followed by the UK and The Netherlands (Table 1). The country with the lowest mean AA intake in both sexes was Italy. In general, men had higher mean AA intake than women (31.90 μg/day and 0.40 μg/kg body weight/day versus 23.81 μg/day and 0.37 μg/kg body weight/day, respectively). In the full EPIC cohort, mean AA intake was 26.22 μg/day (0.38 μg/kg body-weight/day) with a standard deviation of 14.79 μg/day (0.21 μg/kg body-weight/day), and the 10th–90th percentile range was 10.25–45.89 μg/day (0.15–0.66 μg/kg body-weight/day).

The baseline characteristics of total AA intake and covariates used in the analyses are shown in supplementary Table S1, available at *Annals of Oncology* online.

Overall, AA intake was not associated with PC risk (Table 2). The HR and 95% CI for BMI (kg/m²) and PC in this model was 1.02 (1.00–1.03). Furthermore, when 87 PC cases diagnosed during the first 2 years of follow-up were excluded from the analysis, no association was found between AA intake and PC

(Table 2). When the analysis was restricted to microscopically confirmed cases ($n = 608$), HRs were slightly higher than the null value, but were not statistically significant (Table 2).

Subgroup analyses were stratified by smoking status, sex, diabetes, EPIC country, BMI, WHR, and baseline alcohol intake. HRs for AA intake and PC risk in never smokers were similar to HR estimates in ever smokers (Table 2). There was statistically significant heterogeneity by alcohol intake (interaction P value 0.02), but not by sex or diabetes status at baseline (interaction P value 0.23 and 0.56, respectively). No statistically significant heterogeneity in the relation between AA intake and PC risk was observed between countries classified by geographic location or by AA intake level (all P values >0.23, data not shown).

When the BMI was classified by WHO cut points, the underweight subgroup was analyzed with the normal weight group due to the small sample size. A test for interaction between AA and BMI was statistically significant (interaction P value 0.02). In 64 039 obese participants, AA intake was inversely associated with PC risk (trend test P value 0.0037), although this result was based only on 144 obese PC cases. No associations between AA intake and PC risk were observed in overweight or normal/underweight participants (Table 2).

Potential confounders of the AA and PC relation in obese participants were evaluated. Differences, in terms of confounding factors, between the entire cohort and the obese subgroup were negligible (data not shown). The inverse association between AA intake and PC in obese persons by smoking status (never versus ever) was similar to the inverse association observed in all obese persons (data not shown).

Stratified analyses by WHR in quartiles (0.35–0.76, 0.77–0.83, 0.84–0.91, 0.92–1.92), waist circumference in tertiles (<78 cm, 78–91 cm, >91 cm), body weight in tertiles (<63 kg, 63–75 kg, >75 kg), and height in tertiles (<161 cm, 161–169 cm, >169 cm) were also carried out. The results obtained did not indicate effect measure modification by these variables (data not shown).

Sensitivity analyses of the association between AA intake and PC risk in obese participants were carried out. The Goldberg criteria variable was added to Cox models with continuous AA intake (per 10 μg), and showed a statistically significant inverse association (HR: 0.73, 95% CI: 0.60–0.89). Second, the analysis was restricted to obese participants with plausible energy intake (81 cases). Although HRs for AA intake quintiles and PC risk were below unity, they were no longer statistically significant; whereas the continuous variable (per 10 μg/day) still showed a statistically significant inverse association (HR: 0.78, 95% CI: 0.61–0.98).

We also excluded obese persons from countries and centers that collected self-reported data for height and weight and found no differences (data not shown).

discussion

This study found no overall association between questionnaire-based dietary intake of AA and PC risk, even after exclusion of cases diagnosed within the first 2 years of follow-up, and when restricting the analysis to microscopically confirmed cases. We observed suggestive evidence for heterogeneity of the

Table 2. Hazard ratios (HRs) and 95% confidence intervals (CIs) for dietary intake of acrylamide (AA) and pancreatic cancer (PC) risk in EPIC

	AA intake per 10 µg/day	AA intake in quintiles (µg/day)					P for interaction
		0–14.09	14.10–20.14	20.15–26.91	26.92–37.08	37.09–261.36	
Final model							
n cases	865	156	157	155	168	229	
HR (95% CI) ^a	0.95 (0.89–1.01)	1.00 (ref)	0.90 (0.71–1.15)	0.78 (0.60–1.01)	0.68 (0.52–0.90)	0.77 (0.58–1.04)	–
Cases diagnosed ≥2 years after recruitment							
n cases	778	137	139	149	146	207	
HR (95% CI) ^a	0.95 (0.88–1.02)	1.00 (ref)	0.89 (0.69–1.15)	0.83 (0.64–1.09)	0.66 (0.49–0.88)	0.78 (0.57–1.07)	–
Microscopically confirmed cases ^b							
N cases	608	96	113	116	113	170	
HR (95% CI) ^a	1.00 (0.88–1.14)	1.00 (ref)	0.99 (0.54–1.79)	1.15 (0.61–2.17)	1.21 (0.62–2.38)	1.10 (0.55–2.24)	–
Never smokers							
n cases	315	62	62	62	58	71	0.88
HR (95% CI) ^c	0.94 (0.84–1.06)	1.00 (ref)	0.89 (0.61–1.31)	0.82 (0.55–1.23)	0.68 (0.43–1.06)	0.86 (0.52–1.41)	
Ever smokers ^d							
n cases	522	91	93	86	103	149	
HR (95% CI) ^c	0.96 (0.88–1.04)	1.00 (ref)	0.92 (0.67–1.27)	0.71 (0.51–1.01)	0.67 (0.47–0.96)	0.75 (0.51–1.11)	
Women							
n cases	469	112	110	86	83	78	0.10
HR (95% CI) ^e	0.92 (0.83–1.01)	1.00 (ref)	0.89 (0.66–1.18)	0.68 (0.49–0.93)	0.60 (0.42–0.86)	0.67 (0.45–1.00)	
Men							
n cases	396	44	47	69	85	151	
HR (95% CI) ^e	0.96 (0.88–1.05)	1.00 (ref)	0.96 (0.61–1.52)	1.05 (0.67–1.66)	0.89 (0.56–1.42)	0.99 (0.60–1.61)	
Normal & Underweight ^f							
n cases	358	59	72	65	79	83	0.02
HR (95% CI) ^g	0.91 (0.81–1.01)	1.00 (ref)	0.99 (0.68–1.44)	0.77 (0.51–1.15)	0.79 (0.52–1.20)	0.72 (0.45–1.16)	
Overweight ^h							
n cases	363	53	61	67	69	113	
HR (95% CI) ^g	1.05 (0.95–1.15)	1.00 (ref)	1.10 (0.73–1.65)	1.09 (0.72–1.67)	0.90 (0.57–1.41)	1.26 (0.78–2.02)	
Obese ⁱ							
n cases	144	44	24	23	20	33	
HR (95% CI) ^g	0.73 (0.61–0.88)	1.00 (ref)	0.54 (0.31–0.93)	0.44 (0.24–0.78)	0.26 (0.13–0.50)	0.32 (0.16–0.63)	

EPIC, European Prospective Investigation into Cancer and Nutrition; HR, hazards ratio; CI, confidence interval; BMI, body mass index.

^aStratified by age at recruitment and center. Adjusted for sex, total energy intake (per 1000 kcal/day), smoking intensity, diabetes, alcohol intake, and BMI (g/day).

^bCytology or hematology, histology/cytology of metastasis, histology/cytology of primary tumor, and autopsy.

^cStratified by age at recruitment and center. Adjusted for sex, total energy intake (per 1000 kcal/day), diabetes, alcohol intake, and BMI.

^dEver smokers: former and current smokers.

^eStratified by age at recruitment and center. Adjusted for total energy intake (per 1000 kcal/day), smoking intensity, diabetes, alcohol intake, and BMI.

^fNormal weight and underweight: <25 kg/m² (underweight <18.5 kg/m², *n* cases = 5).

^gStratified by age at recruitment and center. Adjusted for sex, total energy intake (per 1000 kcal/day), smoking intensity, diabetes, and alcohol intake.

^hOverweight: ≥25 to <30 kg/m².

ⁱObese: ≥30 kg/m².

association between AA intake and PC risk by BMI; however, when stratified analyses were carried out using the waist and hip circumference or WHR there was no evidence for heterogeneity.

The results presented in this study are in line with previous studies based on food intake questionnaire data and PC risk [13–15]. An Italian case–control study found no statistically significant associations between AA intake and PC, but reported elevated ORs for some AA quintiles. Both the NLCS and the ATBC cohort studies concluded that AA intake was not associated with PC risk.

Similar to our study, an inverse association between dietary intake of AA and PC risk was reported in the Italian case–control study, when the analysis was restricted to obese

participants, but specific ORs were not reported [15]. In contrast with EPIC results, an elevated relative risk for PC of 1.59 for an increase of 10 µg/day of AA in obese participants (based on 14 cases) was observed in the NLCS study [13].

BMI is widely used in epidemiology; however, misclassification of participants may occur because muscularity and bone weight are not accounted for. For this reason, other indices of fat accumulation such as waist circumference, hip circumference, and their ratio (WHR) were also evaluated [23]. Nevertheless, it is worth noting that the BMI measures overall body fatness, whereas the waist and hip circumference and WHR are measures of abdominal fatness. When we used these variables instead of BMI, we did not observe any inverse

association between AA intake and PC risk. In EPIC, the waist and hip circumference and WHR were better predictors of PC risk than the BMI [24]. In a pooled analysis of cohort studies of PC (Panscan), the BMI was a statistically significant risk factor for PC risk, whereas the WHR was more apparent in women than men (only the highest quartile was statistically significant) [25]. Therefore, the inverse association between AA intake and PC risk in obese persons must be interpreted with caution.

The major strength of the EPIC cohort is the prospective collection of exposure and diet information, meaning that recall bias is unlikely since exposure information was collected years before cancer diagnosis. Furthermore, the number of PC cases in our study was considerably higher than in the studies published to date: the Italian case-control study analyzed 326 cases [15], the NLCS study 349 cases [13], and the ATBC study 192 cases [14].

There are some weaknesses in our study. Occupational exposures were not included in the analysis since detailed occupational histories are not available in EPIC. Some food questionnaires used in EPIC centers were not specifically designed to collect information on cooking temperature and cooking methods which have been shown to influence AA levels in foods. Therefore, there may have been some misclassification of exposure because of imperfect dietary assessment methods, and further, dietary reporting errors have been shown to be associated with BMI in EPIC [26]. In addition, we cannot rule out the potential for residual confounding in our analyses, the possibility that some foods that contribute to AA exposure in EPIC were not assessed, and that AA was discovered in food in 2002 (after the time of recruitment). Further, the AA content may vary greatly within the same food items. In order to reduce some variability in questionnaire-based AA intake assessment, all multivariable models were adjusted for total energy intake since some studies have reported that the validity of dietary AA exposure assessment improves after adjustment for total energy consumption [27, 28]. Moreover, the correlation coefficient between AA intake based on food intake questionnaires and on a single 24HDR in EPIC was rather low at 0.17 [28]. This suggests that there may have been errors in our estimates of AA intake, but could also indicate that a single 24HDR is insufficient to assess the dietary intake of AA. Biomarker studies may help to address some of these issues.

In conclusion, the results from this study indicate that dietary intake of AA is not associated with an increased risk of PC in the EPIC cohort. Future research on AA and health with more valid and reliable estimates of dietary AA as well as biomarkers of internal dose should be considered.

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disclosure

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references

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; 63: 11–30.
2. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. *Eur J Cancer* 2010; 46: 765–781.
3. Anderson KE, Mack TM, Silverman DT. Cancer of the pancreas. In Schottenfeld D, Fraumeni JF, Jr (eds), *Cancer Epidemiology and Prevention*, 3rd edition. New York: Oxford University Press 2006; 721–762.
4. Iodice S, Gandini S, Maisonneuve P et al. Tobacco and the risk of pancreatic cancer: a review and meta-analysis. *Langenbecks Arch Surg* 2008; 393: 535–545.
5. Yeo TP, Lowenfels AB. Demographics and epidemiology of pancreatic cancer. *Cancer J* 2012; 18: 477–484.
6. IARC. IARC working group on the evaluation of carcinogenic risks to humans: some industrial chemicals. Lyon, 15–22 February 1994. *IARC Monogr Eval Carcinog Risks Hum* 1994; 60: 1–560.
7. Tareke E, Rydberg P, Karlsson P et al. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem* 2002; 50: 4998–5006.
8. Bergmark E. Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and nonsmokers. *Chem Res Toxicol* 1997; 10: 78–84.
9. Vesper HW, Slimani N, Hallmans G et al. Cross-sectional study on acrylamide hemoglobin adducts in subpopulations from the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. *J Agric Food Chem* 2008; 56: 6046–6053.
10. Dybing E, Farmer PB, Andersen M et al. Human exposure and internal dose assessments of acrylamide in food. *Food Chem Toxicol* 2005; 43: 365–410.
11. Freisling H, Moskal A, Ferrari P et al. Dietary acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition differs greatly according to geographical region. *Eur J Nutr* 2013; 52: 1369–1380.
12. Hogervorst JG, Baars BJ, Schouten LJ et al. The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. *Crit Rev Toxicol* 2010; 40: 485–512.
13. Hogervorst JG, Schouten LJ, Konings EJ et al. Dietary acrylamide intake is not associated with gastrointestinal cancer risk. *J Nutr* 2008; 138: 2229–2236.

14. Hirvonen T, Kontto J, Jestoi M et al. Dietary acrylamide intake and the risk of cancer among Finnish male smokers. *Cancer Causes Control* 2010; 21: 2223–2229.
15. Pelucchi C, Galeone C, Talamini R et al. Dietary acrylamide and pancreatic cancer risk in an Italian case–control study. *Ann Oncol* 2011; 22: 1910–1915.
16. Riboli E, Hunt KJ, Slimani N et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* 2002; 5: 1113–1124.
17. Slimani N, Ferrari P, Ocke M et al. Standardization of the 24-hour diet recall calibration method used in the European Prospective Investigation into Cancer and Nutrition (EPIC): general concepts and preliminary results. *Eur J Clin Nutr* 2000; 54: 900–917.
18. Haftenberger M, Lahmann PH, Panico S et al. Overweight, obesity and fat distribution in 50- to 64-year-old participants in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public Health Nutr* 2002; 5: 1147–1162.
19. Wareham NJ, Jakes RW, Rennie KL et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Public Health Nutr* 2003; 6: 407–413.
20. Wilson KM, Balter K, Adami HO et al. Acrylamide exposure measured by food frequency questionnaire and hemoglobin adduct levels and prostate cancer risk in the cancer of the prostate in Sweden study. *Int J Cancer* 2009; 124: 2384–2390.
21. Schoenfeld D. Partial residuals for the proportional hazards regression model. *Biometrika* 1982; 69: 239–241.
22. Goldberg GR, Black AE, Jebb SA et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr* 1991; 45: 569–581.
23. Nishida C, Ko GT, Kumanyika S. Body fat distribution and noncommunicable diseases in populations: overview of the 2008 WHO Expert Consultation on waist circumference and waist–hip ratio. *Eur J Clin Nutr* 2010; 64: 2–5.
24. Berrington DG, Spencer EA, Bueno-de-Mesquita HB et al. Anthropometry, physical activity, and the risk of pancreatic cancer in the European prospective investigation into cancer and nutrition. *Cancer Epidemiol Biomarkers Prev* 2006; 15: 879–885.
25. Arslan AA, Helzlsouer KJ, Kooperberg C et al. Anthropometric measures, body mass index, and pancreatic cancer: a pooled analysis from the Pancreatic Cancer Cohort Consortium (PanScan). *Arch Intern Med* 2010; 170: 791–802.
26. Freisling H, van Bakel MM, Biessy C et al. Dietary reporting errors on 24 h recalls and dietary questionnaires are associated with BMI across six European countries as evaluated with recovery biomarkers for protein and potassium intake. *Br J Nutr* 2012; 107: 910–920.
27. Schatzkin A, Kipnis V. Could exposure assessment problems give us wrong answers to nutrition and cancer questions? *J Natl Cancer Inst* 2004; 96: 1564–1565.
28. Ferrari P, Freisling H, Duell EJ et al. Challenges in estimating the validity of dietary acrylamide measurements. *Eur J Nutr* 2012 November 1 [epub ahead of print], doi: 10.1007/s00394-012-0457-7.

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Family history of cancer and the risk of cancer: a network of case–control studies

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Background: The risk of many cancers is higher in subjects with a family history (FH) of cancer at a concordant site. However, few studies investigated FH of cancer at discordant sites.

Patients and methods: This study is based on a network of Italian and Swiss case–control studies on 13 cancer sites conducted between 1991 and 2009, and including more than 12 000 cases and 11 000 controls. We collected information on history of any cancer in first degree relatives, and age at diagnosis. Odds ratios (ORs) for FH were calculated by multiple logistic regression models, adjusted for major confounding factors.

Results: All sites showed an excess risk in relation to FH of cancer at the same site. Increased risks were also found for oral and pharyngeal cancer and FH of laryngeal cancer (OR = 3.3), esophageal cancer and FH of oral and pharyngeal

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Dietary intake of acrylamide and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort

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Background: Three prospective studies have evaluated the association between dietary acrylamide intake and endometrial cancer (EC) risk with inconsistent results. The objective of this study was to evaluate the association between acrylamide intake and EC risk: for overall EC, for type-I EC, and in never smokers and never users of oral contraceptives (OCs). Smoking is a source of acrylamide, and OC use is a protective factor for EC risk.

Methods: Cox regression was used to estimate hazard ratios (HRs) for the association between acrylamide intake and EC risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Acrylamide intake was estimated from the EU acrylamide monitoring database, which was matched with EPIC questionnaire-based food consumption data. Acrylamide intake was energy adjusted using the residual method.

Results: No associations were observed between acrylamide intake and overall EC ($n=1382$) or type-I EC risk ($n=627$). We observed increasing relative risks for type-I EC with increasing acrylamide intake among women who both never smoked and were non-users of OCs (HR_{Q5vsQ1} : 1.97, 95% CI: 1.08–3.62; likelihood ratio test (LRT) P -value: 0.01, $n=203$).

Conclusions: Dietary intake of acrylamide was not associated with overall or type-I EC risk; however, positive associations with type I were observed in women who were both non-users of OCs and never smokers.

Acrylamide is a known neurotoxin in humans, and a carcinogen in animals (Friedman, 2003; LoPachin and Gavin, 2008; Hogervorst *et al*, 2010). In 1994, based on animals studies, as well as evidence found in humans, the International Agency for Research on Cancer (IARC) classified acrylamide as 'probably carcinogenic' to humans (IARC group 2A; IARC, 1994). In 2002, Swedish researchers discovered acrylamide in some heat-treated carbohydrate-rich foods (Tareke *et al*, 2002), and further research concluded that acrylamide is formed during common cooking procedures (predominantly through the Maillard reaction), such as frying, grilling, and baking (Friedman, 2003). In the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, the main determinants of estimated dietary intake of acrylamide based on 24-h dietary recall (DR) were bread, crisp bread, rusks, coffee, fried potatoes, cakes, biscuits, and cookies (Freisling *et al*, 2013). Acrylamide is also a component of cigarette smoke, thus, smoking is an important source of exposure (Boettcher *et al*, 2005; Vesper *et al*, 2008).

Acrylamide is metabolised via the Cyp2e1 enzyme system to glycidamide, a chemically reactive epoxide and mutagen in animals (Doroshenko *et al*, 2009; Hogervorst *et al*, 2010). After acrylamide administration, hormone-related (including uterine tumours) and other tumours (e.g., oral tissues) have been observed in rats (Johnson *et al*, 1986).

Endometrial cancer (EC) is the fourth most common cancer diagnosed in European women, but mortality is relatively low with a 5-year survival rate varying from 65 to 85% (Cook *et al*, 2006; Ferlay *et al*, 2013). There is considerable international variation in incidence as well as mortality, and both rates increase dramatically with age (Cook *et al*, 2006; Ferlay *et al*, 2013; Jamison *et al*, 2013). Established risks factors for EC are obesity, low physical activity, history of polycystic ovary syndrome, and greater lifetime exposure to estrogens (Kaaks *et al*, 2002; Cook *et al*, 2006). The use of oral contraceptives (OCs, containing both oestrogen and progestin in the formula) is well established to lower the risk of developing EC (Cook *et al*, 2006; Gierisch *et al*, 2013). There is evidence that tobacco smoking also reduces the risk of EC (Terry *et al*, 2004; Cook *et al*, 2006); however, an EPIC study reported an increased risk of EC in premenopausal women who smoked (Al-Zoughool *et al*, 2007). Endometrial cancer is generally classified into two types: type-I EC are mostly endometrioid adenocarcinomas and are associated with unopposed oestrogen exposure; and type-II EC tumours are mainly serous carcinomas, are believed to be oestrogen independent, and have poor prognosis (Amant *et al*, 2005; Setiawan *et al*, 2013).

Three prospective epidemiological studies have assessed the relationship between dietary intake of acrylamide and EC risk. The Netherlands Cohort Study (NLCS) observed a positive association

between acrylamide intake and EC risk, especially in never smokers (Hogervorst *et al*, 2007). Likewise, the Nurses' Health Study (NHS) reported an increased relative risk among women with the highest acrylamide intake (Wilson *et al*, 2010); however, no associations between acrylamide intake and EC were observed in the Swedish Mammography Cohort (SMC; Larsson *et al*, 2009).

The present study evaluated the association between questionnaire-based dietary intake of acrylamide and the risk of overall EC (type I, type II, and undefined) and type-I EC tumours, using data from 301 113 EPIC cohort participants. Subgroup analyses among never-smoking women and never users of OCs were performed with the aim to eliminate the influence of smoking (both a source of acrylamide and a protective factor) and the protective effect of OCs on EC risk.

METHODS

Study population. The EPIC study was initiated between 1992 and 1998 in 23 centres from 10 European countries with the aim to investigate the relationships between nutrition and lifestyle factors, and cancer and other chronic diseases. All participants gave written informed consent. Ethical review boards from the IARC and local centres participating in EPIC approved the study. The EPIC methodology has been reported in detail by Riboli *et al* (2002).

The EPIC study includes 521 330 participants, of which 367 903 are women. A total of 66 790 women were excluded from the current analyses because they were diagnosed with cancer before recruitment ($n = 19\,853$), had a hysterectomy ($n = 35\,116$), had incomplete follow-up data ($n = 2896$), had no lifestyle or dietary information ($n = 2877$), and no information on dietary intake of acrylamide at baseline ($n = 3$), or had an extreme ratio of energy intake to energy required ($n = 6045$); resulting in 301 113 participants for this analysis.

Identification of endometrial cancer cases. Information on cancer incidence was obtained through population cancer registries, or via a combination of methods: health insurance records, cancer and pathology registries, and active follow-up (France, Germany, Naples, and Greece). Subjects were followed until cancer diagnosis (except non-melanoma skin cancer), emigration, death, or until the end of follow-up (dates varied between centres, from December 2004 to June 2010).

Tumour morphology was specified for 664 (48%) cases, of which 627 (93%) were classified as type I (endometrioid adenocarcinomas), and 37 (7%) as type II (serous, or clear cell, or squamous adenocarcinomas; Tavassoli and Devilee, 2003). Overall EC comprises type I, type II, and cases that were undefined for histology. Tumours were classified as C54 according to the International Classification of Diseases, 10th revision.

Dietary and acrylamide intake assessment. Information on diet was assessed at baseline (with timeframe referring to the previous 12 months) through country-specific, validated dietary questionnaires (DQ; Riboli *et al*, 2002). The development of the acrylamide database in EPIC has been previously described (Freisling *et al*, 2013; Obon-Santacana *et al*, 2013). To summarise, the EPIC acrylamide database is a compilation of the information acquired to a large extent from the European Community Institute for Reference Materials and Measurements (IRMM). The average acrylamide levels for specific foods in the IRMM database were obtained through a combination of methods based on either liquid or gas chromatography coupled to mass spectrometry. All food items with acrylamide data derived from the IRMM database were classified according to EPIC-Soft food classification (Voss *et al*, 1998; Slimani *et al*, 2000). The reported foods on the DQ and, when available, their relevant description (e.g., baked potatoes) were matched with the corresponding foods in the acrylamide

database. Information on cooking methods for acrylamide sources was available for potatoes (except in Italy), bread, and breaded meats. If an exact match was not possible, the food was linked to the mean of all foods of the respective food group in the acrylamide database (Freisling *et al*, 2013; Obon-Santacana *et al*, 2013).

Lifestyle and reproductive information assessment. At baseline, questionnaires were used to collect data on tobacco smoking, education, physical activity, and menstrual and reproductive factors (i.e., age at first menstrual period, ever use of OCs, ever use of hormone replacement therapy (HRT)). Baseline menopausal status was self-reported for each woman in most centres, and in case of incomplete data, an algorithm was developed based on the age at recruitment: women were classified as premenopausal if their baseline ages were <46 years, or reported having menstrual cycles the year before recruitment; perimenopausal if their ages were between 46 and 55 years, or had irregular menses the year before recruitment; and postmenopausal if their ages were >56 years, or had bilateral ovariectomy (surgical menopause), or had <4 menstrual cycles in the past year before recruitment (Riboli *et al*, 2002).

Height, weight, and waist or hip circumference were measured at baseline by trained personnel for all EPIC participants, except for most participants in France, Norway and Oxford cohorts, where height and weight were self-reported. Umeå and Norway did not record data on waist or hip circumference, and only some participants from France have information on waist (29%) and hip circumference (29%; Riboli *et al*, 2002).

Statistical analysis. Proportional hazards models (Cox regression) were used to estimate hazards ratio (HR) and 95% confidence intervals (95% CI) for overall EC risk in relation to dietary intake of acrylamide. Analyses were also performed separately for risk of type-I EC. Analyses for type-II EC cases were not carried out due to small sample sizes ($n = 37$). All multivariate models had age as the time scale and were stratified by study centre to control for centre effects (i.e., questionnaire design and follow-up procedures), and by age at recruitment (in 1-year categories) as the primary time variable.

All estimates of acrylamide intake in these analyses were energy adjusted using the residual method (Willett, 1998; Ferrari *et al*, 2013). One continuous variable and one categorical variable for dietary intake of acrylamide were evaluated in Cox models: average daily intake in $10\,\mu\text{g}$ increments ($10\,\mu\text{g}$ per day), and quintiles of intake (μg per day) based on the distribution in the full EPIC cohort of women.

The following variables were included as known risk factors or potential confounders in these analyses: body mass index (BMI, kg m^{-2}), smoking status (never smokers, current pipe or cigar or occasional smokers, current cigarette smokers: 1–15, 16–25, or ≥ 26 cigarettes per day, former cigarette smokers who quit >20 years, 11–20 years, or ≤ 10 years before recruitment), history of diabetes (no, yes), OC use (never, ever), HRT use (never, ever), baseline menopause status combined with age at menopause (premenopausal, perimenopausal, postmenopausal with: <45 , 45–49, 50–52, 53–55, and ≥ 56 years, surgical menopause, postmenopausal women with missing age at menopause), parity (nulliparous, 1, 2, ≥ 3 , parous but with missing number of full-term pregnancies), and age at menarche (<12 , 12, 13, 14, and ≥ 15 years). Variables for education level (none, primary, technical/professional, secondary, and higher education), physical activity using the Cambridge index (Wareham *et al*, 2003), alcohol intake (non-drinkers, drinkers of 0–6, >6 –12, >12 –24, and $>24\,\text{g}$ per day), total fat (g per day), total fibre (g per day), vegetables (g per day), and fruits, nuts and seeds consumption (g per day) were evaluated, but were not included in final models because they did not change effect estimates $>10\%$. Missing values for specific variables were categorised as 'unknown' and were included in the

analyses. All statistical models presented in this study were further adjusted for total energy intake (per 1000 kcal per day).

Analyses of effect-measure modification were carried out by known EC risk factors (BMI, menopausal status, and HRT use), by known protective factors (OC use, and smoking status), by geographical region, and by factors that may affect the activity of Cyp2e1 (alcohol intake, and BMI; Wilson *et al.*, 2009; Freisling *et al.*, 2013). The following subgroups were examined: BMI ($< 25 \text{ kg m}^{-2}$, $\geq 25 \text{ kg m}^{-2}$), OC use (never, ever), HRT use (never, ever), baseline menopausal status (premenopausal, perimenopausal, and postmenopausal), smoking status (never, current, or former smokers), and alcohol intake (never, ever drinkers). For region-specific analyses, countries were classified as northern (France, UK, The Netherlands, Germany, Sweden, Denmark, and Norway) and southern (Italy, Spain, and Greece); and by median acrylamide-intake level ('high' $\geq 21 \mu\text{g}$ per day and 'low' $< 21 \mu\text{g}$ per day) in the EPIC cohort.

Sensitivity analyses were additionally performed excluding all cases diagnosed during the first 2 years of follow-up, with the aim to avoid possible influences of preclinical disease on dietary habits including intakes of acrylamide.

To evaluate dose-response trends, the median value for each acrylamide quintile was estimated and included in a score test. Statistical significance of effect-measure modification was evaluated using a LRT and based on the continuous acrylamide intake variable. The proportional hazards (PHs) assumption was tested in STATA (College Station, Texas, USA) using Schoenfeld residuals (Schoenfeld, 1982), and it was met for type-I EC analyses; however, it was violated for overall EC analyses. Variables responsible for the PH violation were: OC use, HRT use, and smoking status; thus, stratified analyses by these variables were also performed for overall EC risk, and the PH assumption was subsequently met. All analyses were performed using SAS v. 9.1 (Cary, NC, USA).

RESULTS

Basic information on cohorts members. The average acrylamide intake in the EPIC subcohort of women was $24 \pm 13 \mu\text{g}$ per day

($0.4 \pm 0.2 \mu\text{g}$ per kg body weight per day), and the 10th–90th percentile range was 10–41 μg per day (0.2 – $0.6 \mu\text{g}$ per kg body weight per day). Denmark, followed by the UK and The Netherlands, had the highest mean and median dietary acrylamide intakes, while Italy had the lowest acrylamide intake (Table 1). In total, after 11 years of follow-up there were 1382 first primary EC cases, of which 627 were classified as type-I EC, 37 type-II EC, and 718 cases that were not specified with regard to histology (Table 1).

Women with the highest acrylamide-intake levels tended to have the highest intakes of energy, total fats, total carbohydrates, vegetables, and coffee. Women with the highest intake levels tended to be premenopausal, have a higher proportion of OC use and with longer duration, and were more often current smokers or former smokers at baseline (Table 2). In contrast, women classified in the lower quintiles tended to be postmenopausal, non-consumers of alcohol and tobacco, and to have lower levels of physical activity (Table 2). There were few differences across acrylamide intake quintiles by age, age at first menstrual period, age at menopause, BMI, or waist-to-hip ratio (Table 2).

Overall EC risk and type-I EC risk. No association was observed between acrylamide intake and overall EC (Table 3) or type-I EC risk (Table 4). Similar results were found when we restricted the analyses to cases diagnosed 2 years after recruitment (Tables 3 and 4), or when known type-I and type-II EC were combined in the same analysis (data not shown). Further, an analysis among EC cases that could not be classified into type-I or type-II EC was also carried out, but no associations were observed (data not shown). Most of the stratified analyses performed with overall EC (type I, type II, and undefined) cases indicated no heterogeneity between subgroups (Table 3). When stratified analyses by OC use, and by OC use and smoking were performed, statistically significant LRT *P*-values were observed; however, neither the continuous nor the categorical acrylamide variable suggested an association with disease risk (Table 3).

Effect-measure modification by OC use and smoking in type-I EC. Subgroup analyses for known type-I EC were also stratified by smoking status, OC use, menopausal status, HRT use, BMI, and geographical region. None of the HRs in never smokers or ever

Table 1. Estimated dietary intake of acrylamide and EC cases by country in the EPIC subcohort of women

Country	Cohort sample	Person-years	EC cases N (%)	Type-I cases N (%)	Type-II cases N (%)	Cases undefined by type N (%)	Acrylamide (μg per day) Mean \pm s.d.	Acrylamide ^a (μg per day) Mean \pm s.d.	Acrylamide (μg per kg body weight per day) Mean \pm s.d.
France	60 702	629 899	276 (20.0)	79 (12.6)	3 (8.1)	194 (27.0)	20.4 \pm 8.8	18.3 \pm 6.6	0.4 \pm 0.2
Italy	27 760	310 816	132 (9.6)	48 (7.7)	1 (2.7)	83 (11.6)	10.9 \pm 6.1	8.8 \pm 5.7	0.2 \pm 0.1
Spain	22 783	275 042	102 (7.4)	48 (7.7)	3 (8.1)	51 (7.1)	20.6 \pm 12.1	21.3 \pm 10.3	0.3 \pm 0.2
United Kingdom	46 068	513 816	170 (12.3)	74 (11.8)	5 (13.5)	91 (12.7)	33.1 \pm 15.3	33.4 \pm 13.1	0.5 \pm 0.3
The Netherlands	22 140	260 499	107 (7.7)	59 (9.4)	5 (13.5)	43 (6.0)	31.2 \pm 13.7	31.7 \pm 12.1	0.5 \pm 0.2
Greece	13 967	136 097	18 (1.3)	4 (0.6)	1 (2.7)	13 (1.8)	19.2 \pm 9.1	19.8 \pm 7.2	0.3 \pm 0.1
Germany	23 321	231 579	82 (5.9)	67 (10.7)	4 (10.8)	11 (1.5)	24.5 \pm 11.2	25.3 \pm 9.7	0.4 \pm 0.2
Sweden	26 375	349 308	183 (13.2)	1 (0.2)	4 (10.8)	178 (24.8)	22.4 \pm 9.7	23.6 \pm 8.2	0.3 \pm 0.2
Denmark	24 473	269 910	182 (13.2)	140 (22.3)	9 (24.3)	33 (4.6)	35.6 \pm 11.7	35.5 \pm 10.2	0.5 \pm 0.2
Norway	33 524	326 296	130 (9.4)	107 (17.1)	2 (5.4)	21 (2.9)	17.9 \pm 6.5	20.6 \pm 5.8	0.3 \pm 0.1
Total	301 113	3 303 262	1382	627	37	718	23.7 \pm 13.0	23.7 \pm 12.0	0.4 \pm 0.2

Abbreviations: EC = endometrial cancer; EPIC = European Prospective Investigation into Cancer and Nutrition; s.d. = standard deviation.

^aEnergy adjusted using the residual method.

Table 2. Estimated total dietary intake of acrylamide (energy adjusted using the residual method) and covariates at baseline used in the analyses: EPIC subcohort (301 113 women)

	Energy-adjusted acrylamide intake (μg per day)				
	≤ 14.5	14.6–19.5	19.6–24.2	24.3–32.0	32.1–222.4
Participants (n)	60 222	60 223	60 223	60 223	60 222
Endometrial cancer cases (n)	277	271	298	250	286
Type-I EC cases (n)	105	111	125	122	164
Energy-adjusted acrylamide intake (median; μg per day)	10.7	17.2	21.7	27.4	39.3
Age (years)	51.1 \pm 8.4 ^a	50.8 \pm 9.1	50.1 \pm 9.6	49.7 \pm 10.6	49.6 \pm 11.5
Age at first menstrual period (years) ^b	12.8 \pm 1.5	13.1 \pm 1.5	13.1 \pm 1.5	13.2 \pm 1.5	13.2 \pm 1.6
Age at menopause (years) ^b	49.3 \pm 4.4	49.3 \pm 4.5	49.3 \pm 4.5	49.4 \pm 4.4	49.4 \pm 4.3
Menopausal status (%)					
Premenopausal	36.5	35.76	37.8	40.05	40.15
Perimenopausal	18.16	20.55	19.68	16.51	12.92
Postmenopausal ^c	45.34	43.69	42.52	43.44	46.93
Ever use of OCs (%)					
Yes	49.45	55.8	58.12	61.46	65.48
Unknown	0.65	2.51	4.53	4.04	1.8
Duration of using OCs (years) ^b	6.1 \pm 6.6	7.4 \pm 7.2	7.9 \pm 7.4	8.4 \pm 7.5	8.7 \pm 7.5
Ever use of HRT (%)					
Yes	19.96	22.71	21.94	21.29	22.22
Unknown	3.25	6.69	9.09	9.33	6.37
Duration of using HRT (years) ^b	2.9 \pm 3.1	3.4 \pm 3.3	3.6 \pm 3.6	3.9 \pm 4.2	4.2 \pm 4.6
Smoking status (%)					
Never	59.49	60.01	55.53	52.35	49.68
Former	19.45	20.8	22.71	23.88	25.15
Current	18.86	15.75	18.88	21.61	23.85
Unknown	2.2	3.44	2.88	2.16	1.31
Cigarettes per day (smokers only)	13.1 \pm 8.7	12.5 \pm 7.7	12.8 \pm 7.5	13.2 \pm 7.6	14.0 \pm 7.8
Time since quitting ^d (years)	13.7 \pm 9.0	15.0 \pm 9.6	14.8 \pm 9.8	14.9 \pm 10.1	14.9 \pm 10.5
Prevalent diabetes (%)					
Yes	2.67	2.42	2.0	1.65	1.61
Unknown	1.94	4.42	5.07	4.59	4.64
Alcohol					
Non-consumers (%)	22.56	19.08	16.49	13.51	10.24
Consumers (g per day)	9.2 \pm 14.1	7.2 \pm 10.9	6.6 \pm 10.1	7.6 \pm 10.8	8.5 \pm 10.9
Education (%)					
Primary school completed	31.48	20.23	21.76	21.93	21.13
Higher education ^e	22.57	25.92	23.91	23.56	21.5
Unknown	1.72	2.69	2.98	4.3	6.31
Physical activity (%)					
Inactive	28.99	21.35	19.13	18.26	17.44
Active	9.49	9.71	11.78	15.93	22.08
Unknown	7.09	18.22	19.71	12.13	4.29
BMI (kg m^{-2})	25.1 \pm 4.5	24.6 \pm 4.4	24.7 \pm 4.3	24.8 \pm 4.4	25.0 \pm 4.4
WHR ^b	0.8 \pm 0.1	0.8 \pm 0.1	0.8 \pm 0.1	0.8 \pm 0.1	0.8 \pm 0.1
Energy (kcal)	2098.2 \pm 571.9	1860.1 \pm 521.1	1810.3 \pm 515.9	1873.8 \pm 516.2	2027.5 \pm 523.3
Total fats (g per day)	84.8 \pm 28.3	74.5 \pm 26.3	70.9 \pm 25.8	72.6 \pm 25.9	78.3 \pm 26.4
Carbohydrates (g per day)	224.5 \pm 74.2	203.7 \pm 63.6	204 \pm 62.3	213.0 \pm 63.9	232.7 \pm 67.3
Vegetables (g per day)	252.9 \pm 165.6	232.3 \pm 146.5	203.1 \pm 133.6	198.8 \pm 129.8	204.5 \pm 127.7
Coffee (ml per day)	123.6 \pm 129.9	228.5 \pm 194.4	337.8 \pm 240.2	441.8 \pm 305.9	643.4 \pm 449.3
Bread, crisp bread, and rusks (g per day)	121.1 \pm 76.0	114.9 \pm 65.9	115.7 \pm 66.1	116.6 \pm 67.4	124.2 \pm 69.1
Potatoes (g per day)	48.6 \pm 46.2	70.8 \pm 52.9	84.3 \pm 57.5	95.1 \pm 64.4	105.7 \pm 67.5
Cakes and biscuits (g per day)	34.8 \pm 37.6	34.8 \pm 33.4	38.4 \pm 34.3	42.4 \pm 38.6	48.3 \pm 47.7

Abbreviations: BMI = body mass index; EC = endometrial cancer; EPIC = European Prospective Investigation into Cancer and Nutrition; HRT = hormonal replacement therapy; OCs = oral contraceptives; WHR = waist-to-hip ratio.

^aMean \pm s.d.

^bNumber of women missing the following: age at first menstrual period: 10 321; age at menopause: 201 651; duration of using OCs: 142 462; duration of using HRT: 278 012; number of cigarettes: 243 668; time since quitting smoking: 236 217; and WHR: 88 717.

^cIncludes surgical menopause.

^dOnly in former smokers.

^eHigher education includes any university degree or above.

Table 3. Hazard ratios and 95% confidence intervals for the estimated dietary intake of acrylamide (energy-adjusted using the residual method) and EC risk in EPIC

	Energy-adjusted acrylamide intake (µg per day)							Trend test P-value	LRTP-value ^a
	10 µg increments	Quintiles							
		Q1 (≤14.5)	Q2 (14.6–19.5)	Q3 (19.6–24.2)	Q4 (24.3–32.0)	Q5 (32.1–222.4)			
Final model – overall EC									
N cases HR (95% CI) ^b	1382 0.98 (0.92–1.05)	277 1.00 (ref)	271 1.05 (0.86–1.29)	298 1.11 (0.90–1.36)	250 0.88 (0.71–1.10)	286 0.98 (0.78–1.25)	0.53		
Cases diagnosed ≥2 years after recruitment									
N cases HR (95% CI) ^b	1186 0.98 (0.91–1.05)	240 1.00 (ref)	217 0.97 (0.78–1.20)	268 1.12 (0.89–1.39)	215 0.85 (0.67–1.08)	246 0.95 (0.74–1.23)	0.52		
Overall EC – stratified analyses									
Smoking status									
Never smokers									
N cases HR (95% CI) ^c	747 0.97 (0.89–1.05)	147 1.00 (ref)	142 1.03 (0.79–1.34)	153 1.04 (0.79–1.36)	132 0.82 (0.61–1.10)	173 1.01 (0.75–1.38)	0.90		
Ever smokers ^d								0.20	
N cases HR (95% CI) ^c	587 0.98 (0.89–1.08)	123 1.00 (ref)	118 1.08 (0.80–1.45)	135 1.23 (0.91–1.66)	110 0.96 (0.69–1.33)	101 0.86 (0.60–1.24)	0.23		
OC use									
Non-OC users									
N cases HR (95% CI) ^e	800 1.03 (0.94–1.12)	180 1.00 (ref)	155 1.07 (0.83–1.38)	165 1.09 (0.84–1.42)	127 0.83 (0.62–1.11)	173 1.17 (0.86–1.58)	0.51		
OC users								0.03	
N cases HR (95% CI) ^e	547 0.92 (0.83–1.02)	94 1.00 (ref)	111 1.05 (0.76–1.46)	121 1.16 (0.83–1.61)	117 0.97 (0.68–1.39)	104 0.79 (0.53–1.15)	0.08		
Smoking status combined with OC use									
Never smokers and non-OC users									
N cases HR (95% CI) ^f	477 1.02 (0.92–1.13)	106 1.00 (ref)	90 1.05 (0.76–1.44)	94 1.08 (0.77–1.50)	75 0.82 (0.57–1.18)	112 1.28 (0.88–1.85)	0.24		
Ever smokers ^d and non-OC users									
N cases HR (95% CI) ^f	299 1.02 (0.89–1.17)	68 1.00 (ref)	58 1.09 (0.73–1.65)	68 1.28 (0.84–1.95)	47 0.87 (0.55–1.39)	58 0.98 (0.60–1.60)	0.65		
Never smokers and OC users								0.04	
N cases HR (95% CI) ^f	253 0.89 (0.77–1.03)	39 1.00 (ref)	49 1.03 (0.64–1.67)	52 0.98 (0.60–1.61)	54 0.83 (0.50–1.40)	59 0.73 (0.42–1.26)	0.13		
Ever smokers ^d and OC users									
N cases HR (95% CI) ^f	277 0.93 (0.80–1.08)	54 1.00 (ref)	58 1.10 (0.71–1.69)	63 1.22 (0.78–1.90)	60 1.07 (0.67–1.71)	42 0.76 (0.44–1.30)	0.22		
Alcohol intake									
Never drinkers									
N cases HR (95% CI) ^b	253 1.06 (0.91–1.24)	70 1.00 (ref)	59 0.95 (0.62–1.46)	38 0.72 (0.44–1.18)	35 0.59 (0.35–1.00)	51 1.03 (0.60–1.76)	0.76		
Ever drinkers								0.07	
N cases HR (95% CI) ^b	1129 0.97 (0.90–1.04)	207 1.00 (ref)	212 1.10 (0.87–1.39)	260 1.27 (1.00–1.61)	215 0.96 (0.75–1.24)	235 1.01 (0.77–1.32)	0.54		
Body mass index									
<25 kg m ^{−2}									
N cases HR (95% CI) ^g	1.01 (0.91–1.12)	1.00 (ref)	0.94 (0.70–1.27)	1.13 (0.83–1.53)	0.92 (0.67–1.28)	0.93 (0.64–1.35)	0.68		
≥25 kg m ^{−2}								0.96	
N cases HR (95% CI) ^g	0.99 (0.90–1.08)	1.00 (ref)	1.29 (0.96–1.73)	1.21 (0.89–1.64)	0.94 (0.68–1.31)	1.12 (0.79–1.57)	0.89		

Table 3. (Continued)

Energy-adjusted acrylamide intake (μg per day)								
10 μg increments	Quintiles					Trend test P-value	L RTP-value ^a	
	Q1 (≤ 14.5)	Q2 (14.6–19.5)	Q3 (19.6–24.2)	Q4 (24.3–32.0)	Q5 (32.1–222.4)			
Menopausal status								
Premenopausal								
N cases HR (95% CI) ^b	253 0.88 (0.74–1.04)	67 1.00 (ref)	54 1.12 (0.72–1.74)	52 1.12 (0.70–1.78)	45 1.00 (0.61–1.64)	35 0.68 (0.37–1.22)	0.17	
Perimenopausal								0.05
N cases HR (95% CI) ^b	268 1.05 (0.89–1.23)	51 1.00 (ref)	56 1.08 (0.69–1.70)	73 1.29 (0.82–2.04)	44 0.83 (0.50–1.39)	44 1.18 (0.67–2.10)	0.90	
Postmenopausal ⁱ								
N cases HR (95% CI) ^b	861 1.01 (0.93–1.10)	159 1.00 (ref)	161 1.05 (0.80–1.38)	173 1.06 (0.80–1.40)	161 0.84 (0.62–1.13)	207 1.03 (0.76–1.40)	0.99	

Abbreviations: BMI = body mass index; CI = confidence interval; EC = endometrial cancer; EPIC = European Prospective Investigation into Cancer and Nutrition; HR = hazards ratio; HRT = hormonal replacement therapy; LRT = likelihood ratio test; OCs = oral contraceptives.

^aAll LRT *P*-values for effect measure modification are based on the continuous acrylamide intake variable (per 10 μg per day).

^bStratified by age at recruitment, centre, smoking status, OC use, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, menopause status combined with age at menopause, parity, and age at menarche.

^cStratified by age at recruitment, centre, OC use, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, menopause status combined with age at menopause, parity, and age at menarche.

^dEver smokers: former and current smokers.

^eStratified by age at recruitment, centre, smoking status, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, menopause status combined with age at menopause, parity and age at menarche.

^fStratified by age at recruitment, centre, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, menopause status combined with age at menopause, parity, and age at menarche.

^gStratified by age at recruitment, centre, smoking status, OC use, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), prevalent diabetes, menopause status combined with age at menopause, parity, and age at menarche.

^hStratified by age at recruitment, centre, smoking status, OC use, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, parity, and age at menarche.

ⁱIncludes surgical menopause.

smokers indicated associations between dietary acrylamide intake and type-I EC risk; however, statistically significant evidence for heterogeneity was observed (LRT *P*-value: 0.01; Table 4).

Inverse associations were observed for the highest versus the lowest quintile of acrylamide intake (HR_{Q5vsQ1}: 0.57, 95% CI: 0.34–0.96; *P*-value for trend: 0.01), as well as a continuous variable (HR: 0.83, 95% CI: 0.71–0.95; Table 4). Regarding the HRs obtained in the subgroup of non-OC users, none of them were statistically significant (HR_{10 μg per day}: 1.10, 95% CI: 0.99–1.23; Table 4).

Moreover, the OC-use model was additionally adjusted by duration of OC use (per 2 years of OC use), and the results were similar to those presented without adjustment for this variable (data not shown).

There were some differences in non-dietary variables between OC users and non-users. OC users with the highest acrylamide intake tended to have a higher proportion of former or current smokers, and these women tended to smoke more cigarettes per day than non-users. Further, non-OC users were older than OC users, but with similar age at menopause. With regard to dietary factors, there were no major differences between OC users and non-users (data not shown).

The association between acrylamide intake and type-I EC risk among OC users and non-users was also evaluated by smoking status. Women who at baseline reported being never smokers and non-users of OCs (including 203 type-I EC cases) were at the highest risk of developing type-I EC, when acrylamide was evaluated both as a continuous variable and in quintiles (HR_{10 μg per day}: 1.17, 95% CI: 1.02–1.34; HR_{Q5vsQ1}: 1.97, 95% CI: 1.08–3.62; *P*-value for trend: 0.01; Table 4). Otherwise, associations between dietary acrylamide intake and type-I EC were below the null value in ever smokers (current and former smokers) and OC

users (HR_{10 μg per day}: 0.75, 95% CI: 0.60–0.94; Table 4). The LRT *P*-value of the contrast between ‘never smokers/non-OC users’, ‘ever smokers/non-OC users’, ‘never smokers/OC users’, and ‘ever smokers/OC users’ for the continuous acrylamide intake variable was 0.01 (Table 4).

Other effect-measure modifications in type-I EC. There was no evidence for effect-measure modification by BMI (Table 4), HRT use, or by geographical region (all LRT *P*-values > 0.12, data not shown); however, evidence for effect-measure modification was found when the analyses were stratified by baseline menopausal status (LRT *P*-value: 0.01; Table 4), but none of the individual HRs were statistically significant. Likewise, effect-measure modification was observed by alcohol intake (LRT *P*-value: 0.01), but only the continuous variable in never drinkers showed a statistically significant positive association (HR_{10 μg per day}: 1.23, 95% CI: 1.02–1.47; Table 4).

DISCUSSION

No overall association was observed between dietary intake of acrylamide and overall EC or type-I EC risk; nevertheless, elevated relative risks, as well as *P*-values for linear trend were observed for the association between dietary intake of acrylamide and type-I EC among women who both never smoked and never used OCs. Statistically significant inverse associations between type-I EC risk and acrylamide intake were observed in OC users, and among OC users and ever smokers.

It is widely published that use of OCs (containing oestrogen and progestin) is protective against EC risk, and this effect is

Table 4. Hazard ratios and 95% confidence intervals for the estimated dietary intake of acrylamide (energy-adjusted using the residual method) and type-I endometrial cancer risk in EPIC

		Energy-adjusted acrylamide intake (µg per day)						
		Quintiles						
10 µg increments		Q1 (≤14.5)	Q2 (14.6–19.5)	Q3 (19.6–24.2)	Q4 (24.3–32.0)	Q5 (32.1–222.4)	Trend test P-value	L RTP-value ^a
Final model – Type I								
N cases HR (95% CI) ^b	627 0.98 (0.90–1.07)	105 1.00 (ref)	111 1.00 (0.74–1.35)	125 1.04 (0.77–1.42)	122 0.87 (0.63–1.21)	164 0.97 (0.69–1.36)	0.79	
Cases diagnosed ≥2 years after recruitment								
N cases HR (95% CI) ^b	556 0.96 (0.87–1.06)	98 1.00 (ref)	93 0.89 (0.65–1.23)	117 1.04 (0.76–1.43)	107 0.84 (0.60–1.19)	141 0.93 (0.65–1.32)	0.75	
Type I – stratified analyses								
Smoking status								
Never smokers								
N cases HR (95% CI) ^c	350 1.06 (0.95–1.19)	56 1.00 (ref)	54 0.97 (0.63–1.48)	67 1.14 (0.74–1.74)	69 0.97 (0.62–1.51)	104 1.25 (0.79–1.98)	0.21	
Ever smokers ^d								0.01
N cases HR (95% CI) ^c	257 0.90 (0.78–1.03)	44 1.00 (ref)	51 1.02 (0.64–1.63)	55 1.00 (0.62–1.62)	50 0.80 (0.48–1.34)	57 0.70 (0.41–1.19)	0.09	
OC use								
Non-OC users								
N cases HR (95% CI) ^e	347 1.10 (0.99–1.23)	65 1.00 (ref)	56 0.96 (0.64–1.45)	65 1.09 (0.71–1.67)	58 0.90 (0.57–1.42)	103 1.40 (0.89–2.22)	0.06	
OC users								0.01
N cases HR (95% CI) ^e	273 0.83 (0.71–0.95)	39 1.00 (ref)	54 0.97 (0.62–1.51)	59 0.93 (0.59–1.47)	63 0.79 (0.49–1.28)	58 0.57 (0.34–0.96)	0.01	
Smoking status combined with OC use								
Never smokers and non-OC users								
N cases HR (95% CI) ^f	203 1.17 (1.02–1.34)	35 1.00 (ref)	29 1.03 (0.58–1.81)	36 1.28 (0.72–2.27)	35 1.12 (0.61–2.06)	68 1.97 (1.08–3.62)	0.01	
Ever smokers ^d and non-OC users								
N cases HR (95% CI) ^f	134 1.04 (0.86–1.26)	26 1.00 (ref)	25 0.99 (0.51–1.91)	27 0.99 (0.50–1.98)	21 0.76 (0.36–1.62)	35 1.01 (0.47–2.19)	0.98	
Never smokers and OC users								0.01
N cases HR (95% CI) ^f	145 0.89 (0.73–1.09)	20 1.00 (ref)	25 0.76 (0.40–1.45)	31 0.83 (0.44–1.59)	33 0.68 (0.35–1.35)	36 0.59 (0.29–1.21)	0.17	
Ever smokers ^d and OC users								
N cases HR (95% CI) ^f	120 0.75 (0.60–0.94)	18 1.00 (ref)	25 1.02 (0.52–1.99)	27 1.00 (0.50–1.98)	29 0.84 (0.41–1.72)	21 0.45 (0.20–1.00)	0.02	
Alcohol intake								
Never drinkers								
N cases HR (95% CI) ^b	103 1.23 (1.02–1.47)	28 1.00 (ref)	19 0.76 (0.40–1.44)	13 0.61 (0.29–1.28)	17 0.93 (0.46–1.89)	26 1.77 (0.86–3.64)	0.07	
Ever drinkers								0.01
N cases HR (95% CI) ^b	524 0.93 (0.85–1.03)	77 1.00 (ref)	92 1.09 (0.77–1.54)	112 1.19 (0.83–1.69)	105 0.90 (0.61–1.31)	138 0.91 (0.62–1.35)	0.30	
Body mass index								
<25 kg m ^{−2}								
N cases HR (95% CI) ^g	256 0.86 (0.74–1.00)	43 1.00 (ref)	48 0.88 (0.56–1.38)	62 1.11 (0.71–1.73)	53 0.78 (0.48–1.27)	50 0.56 (0.33–0.96)	0.02	
≥25 kg m ^{−2}								0.28
N cases HR (95% CI) ^g	371 1.06 (0.95–1.18)	62 1.00 (ref)	63 1.12 (0.75–1.69)	63 0.99 (0.64–1.52)	69 0.92 (0.59–1.44)	114 1.34 (0.85–2.10)	0.12	

Table 4. (Continued)

Energy-adjusted acrylamide intake (μg per day)								
	10 μg increments	Quintiles					Trend test P-value	L RTP-value ^a
		Q1 (≤14.5)	Q2 (14.6–19.5)	Q3 (19.6–24.2)	Q4 (24.3–32.0)	Q5 (32.1–222.4)		
Menopausal status								
Premenopausal								
N cases	120	28	25	26	24	17		
HR (95% CI) ^h	0.78 (0.62–0.99)	1.00 (ref)	0.89 (0.48–1.64)	0.91 (0.49–1.71)	0.78 (0.40–1.53)	0.52 (0.24–1.13)	0.09	
Perimenopausal								0.01
N cases	120	24	25	32	20	19		
HR (95% CI) ^h	0.88 (0.70–1.12)	1.00 (ref)	0.77 (0.41–1.43)	0.91 (0.49–1.68)	0.67 (0.33–1.36)	0.59 (0.26–1.31)	0.22	
Postmenopausal ⁱ								
N cases	387	53	61	67	78	128		
HR (95% CI) ^h	1.07 (0.96–1.18)	1.00 (ref)	1.24 (0.81–1.89)	1.25 (0.81–1.95)	1.09 (0.69–1.72)	1.39 (0.88–2.20)	0.17	

Abbreviations: BMI = body mass index; CI = confidence interval; EPIC = European Prospective Investigation into Cancer and Nutrition; HR = hazards ratio; HRT = hormonal replacement therapy; LRT = likelihood ratio test; OCs = oral contraceptives.

^aAll LRT *P*-values for effect measure modification are based on the continuous acrylamide intake variable (per 10 μg per day).

^bStratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, smoking status, prevalent diabetes, OC use, HRT use, menopause status combined with age at menopause, parity, and age at menarche.

^cStratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, OC use, HRT use, menopause status combined with age at menopause, parity, and age at menarche.

^dEver smokers: former and current smokers.

^eStratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, smoking status, prevalent diabetes, HRT use, menopause status combined with age at menopause, parity, and age at menarche.

^fStratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, HRT use, menopause status combined with age at menopause, parity, and age at menarche.

^gStratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), smoking status, prevalent diabetes, OC use, HRT use, menopause status combined with age at menopause, parity, and age at menarche.

^hStratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, smoking status, prevalent diabetes, OC use, HRT use, parity, and age at menarche.

ⁱIncludes surgical menopause.

maintained for years (Amant *et al*, 2005; Cook *et al*, 2006; Cibula *et al*, 2010; Gierisch *et al*, 2013). Likewise, cigarette smoking tends to lower the risk of developing EC, and it is thought to be more pronounced in recent smokers (Cook *et al*, 2006). All the relative risk estimates for type-I EC risk observed among OC users and ever smokers were below the null value; however, because OC use, duration of OC use, and smoking are associated with higher acrylamide intake in EPIC, and are also associated with lower EC risk, residual confounding by these variables may play a role in the observed inverse associations (in OC users and smokers). In addition, OC users, compared to non-OC users, tended to smoke more cigarettes per day and reported less time since having quit smoking. Thus, these baseline characteristics may have partially influenced the results obtained in this subgroup of women. Moreover, it has been hypothesised that acrylamide may have hormonal effects, and the results in non-OC users for type I are potentially compatible with this hypothesis, since type-I EC is considered to be oestrogen driven (Amant *et al*, 2005); nevertheless, this hypothesis has not been substantiated, and other mechanisms (i.e., genotoxicity caused by glycidamide) may be compatible with the results (Hogervorst *et al*, 2010, 2013).

The relation between dietary intake of acrylamide and EC risk has been previously published in three prospective cohort studies. Both the NLCS and NHS studies found statistically significantly increased relative risks: the NLCS among never-smoking women, and the NHS in the entire cohort (Hogervorst *et al*, 2007; Wilson *et al*, 2010). Although the NLCS and NHS studies did not evaluate the association between acrylamide intake and type-I EC specifically, about 80% of EC cases are thought to be type-I endometrioid tumours (Amant *et al*, 2005); thus, the majority of the cases in the previous publications were likely type-I EC cases.

Only the SMC study observed no associations between acrylamide intake and EC risk (Larsson *et al*, 2009), and this could be due to the smaller baseline ranges of acrylamide intake in that study. The median acrylamide intake for the reference group in the SMC was 16.9 μg per day, and for the highest intake category was 32.5 μg per day, whereas in EPIC, the median for the reference group was 9.3 μg per day, and for the highest intake category was 44.0 μg per day. All three previous studies presented statistical models adjusted for OC use, but none reported analyses stratified by OC use.

Some evidence for an inverse association between the highest and lowest acrylamide quintiles and type-I EC risk was observed among women with a BMI $< 25 \text{ kg m}^{-2}$; however, neither the continuous variable for acrylamide intake (per 10 μg per day) nor the LRT P-value were statistically significant. A suggestive increased risk for type-I EC was observed in women who reported never drinking alcohol at baseline when the continuous acrylamide variable was evaluated; nevertheless, this result was based on 103 type-I EC cases. Further, suggestive evidence for heterogeneity of the association between dietary acrylamide intake and type-I EC risk was also indicated by smoking status, and by menopausal status at baseline; nevertheless no dose-response trend was observed.

The strengths of our study are that EPIC is one of the largest prospective cohort studies on diet and cancer, and recall bias is unlikely because exposure and diet information were collected years before cancer diagnoses. The present study had more cases than the other three previously published studies ($n = 1382$), and this allowed us to evaluate known type-I EC separately ($n = 627$). The SMC study analysed 687 EC cases (Larsson *et al*, 2009), the NHS study analysed 484 EC cases (Wilson *et al*, 2010), and the NLCS study evaluated 221 (Hogervorst *et al*, 2007).

The present study had the following limitations: some food preparation techniques (e.g., cooking method) that could have contributed to the variability of total acrylamide intake were not assessed in all EPIC centres. In addition, the correlation coefficient between a single 24-h DR in EPIC, and acrylamide intake derived from food intake questionnaires was low: 0.17 (Ferrari *et al*, 2013). This could indicate that a single 24-h DR may not be enough to accurately estimate the average acrylamide intake. Further, the EPIC acrylamide estimates might have been influenced by measurement error; however, all the analyses were adjusted for energy intake since in EPIC and in other populations, it has been observed that the validity of acrylamide estimates improved after energy intake adjustment (Ferrari *et al*, 2013). Another limitation of our study is that 718 EC cases were not classified in any of the EC subtypes; however, as has been previously mentioned, a large proportion ($\approx 80\%$) of endometrial carcinomas are thought to be type I (Amant *et al*, 2005). Finally, it should be kept in mind that several subgroups have been examined in this study; thus, some of the observed results might be due to chance.

In conclusion, the results of the present study indicate that there were no associations between dietary intake of acrylamide and risk of overall EC or type-I EC; nevertheless, women with elevated acrylamide intake (upper quintile median, 44 μg per day) who both never smoked and never used OCs at baseline, were at higher risk of developing type-I EC relative to women with the lowest intakes. Additional studies with biomarkers of internal dose of acrylamide exposure are needed in order to better understand the associations observed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DISCLAIMER

None of the funding agencies had a role in the design, implementation, analysis or interpretation of study results.

REFERENCES

- Al-Zoughool M, Dossus L, Kaaks R, Clavel-Chapelon F, Tjønneland A, Olsen A, Overvad K, Boutron-Ruault MC, Gauthier E, Linseisen J, Chang-Claude J, Boeing H, Schulz M, Trichopoulou A, Chryssa T, Trichopoulos D, Berrino F, Palli D, Mattiello A, Tumino R, Sacerdote C, Bueno-de-Mesquita HB, Boshuizen HC, Peeters PH, Gram IT, Braaten T, Lund E, Chirlaque MD, Ardanaz E, Agudo A, Larranaga N, Quiros JR, Berglund G, Manjer J, Lundin E, Hallmans G, Khaw KT, Bingham S, Allen N, Key T, Jenab M, Cust AE, Rinaldi S, Riboli E (2007) Risk of endometrial cancer in relationship to cigarette smoking: results from the EPIC study. *Int J Cancer* **121**(12): 2741–2747.
- Amant F, Moerman P, Neven P, Timmerman D, Van LE, Vergote I (2005) Endometrial cancer. *Lancet* **366**(9484): 491–505.
- Boettcher MI, Schettgen T, Kutting B, Pischetsrieder M, Angerer J (2005) Mercapturic acids of acrylamide and glycidamide as biomarkers of the internal exposure to acrylamide in the general population. *Mutat Res* **580**(1–2): 167–176.
- Cibula D, Gompel A, Mueck AO, La VC, Hannaford PC, Skouby SO, Zikan M, Dusek L (2010) Hormonal contraception and risk of cancer. *Hum Reprod Update* **16**(6): 631–650.
- Cook LS, Weiss NS, Doherty JA, Chen C (2006) Endometrial Cancer. In *Cancer Epidemiology and Prevention*, Schottenfeld D, Fraumeni Jr JF (eds) pp 1027–1043. Oxford University Press: New York.
- Doroshenko O, Fuhr U, Kunz D, Frank D, Kinzig M, Jetter A, Reith Y, Lazar A, Taubert D, Kirchheiner J, Baum M, Eisenbrand G, Berger FL, Bertow D, Berkessel A, Sorgel F, Schomig E, Tomalik-Scharte D (2009) In vivo role of cytochrome P450 2E1 and glutathione-S-transferase activity for acrylamide toxicokinetics in humans. *Cancer Epidemiol Biomarkers Prev* **18**(2): 433–443.
- Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JWW, Comber H, Forman D, Bray F (2013) Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur J Cancer* **49**(6): 1374–1403.
- Ferrari P, Freisling H, Duell EJ, Kaaks R, Lujan-Barroso L, Clavel-Chapelon F, Boutron-Ruault MC, Nailler L, Polidoro S, Mattiello A, Palli D, Tumino R, Grioni S, Knuppel S, Tjønneland A, Olsen A, Overvad K, Orfanos P, Katsoulis M, Trichopoulou A, Quiros JR, Ardanaz E, Huerta JM, Etxezarreta PA, Sanchez MJ, Crowe F, Khaw KT, Wareham NJ, Ocke M, Bueno-De-Mesquita B, Peeters PH, Ericson U, Wirfält E, Hallmans G, Johansson I, Engeset D, Nicolas G, Gallo V, Norat T, Riboli E, Slimani N (2013) Challenges in estimating the validity of dietary acrylamide measurements. *Eur J Nutr* **52**(5): 1503–1512.
- Freisling H, Moskal A, Ferrari P, Nicolas G, Knaze V, Clavel-Chapelon F, Boutron-Ruault MC, Nailler L, Teucher B, Grote VA, Boeing H, Clemens M, Tjønneland A, Olsen A, Overvad K, Quiros JR, Duell EJ, Sanchez MJ, Amiano P, Chirlaque MD, Barricarte A, Khaw KT, Wareham NJ, Crowe FL, Gallo V, Oikonomou E, Naska A, Trichopoulou A, Palli D, Agnoli C, Tumino R, Polidoro S, Mattiello A, Bueno-de-Mesquita HB, Ocke MC, Peeters PH, Wirfält E, Ericson U, Bergdahl IA, Johansson I, Hjartaker A, Engeset D, Skeie G, Riboli E, Slimani N (2013) Dietary acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition differs greatly according to geographical region. *Eur J Nutr* **52**(4): 1369–1380.

- Friedman M (2003) Chemistry, biochemistry, and safety of acrylamide. A review. *J Agric Food Chem* **51**(16): 4504–4526.
- Gierisch JM, Coeytaux RR, Peragallo UR, Havrilesky LJ, Moorman PG, Lowery WJ, Dinan M, McBroom AJ, Hasselblad V, Sanders GD, Myers ER (2013) Oral contraceptive use and risk of breast, cervical, colorectal, and endometrial cancers: a systematic review. *Cancer Epidemiol Biomarkers Prev* **22**(11): 1931–1943.
- Hogervorst JG, Baars BJ, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2010) The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. *Crit Rev Toxicol* **40**(6): 485–512.
- Hogervorst JG, Fortner RT, Mucci LA, Tworoger SS, Eliassen AH, Hankinson SE, Wilson KM (2013) Associations between dietary acrylamide intake and plasma sex hormone levels. *Cancer Epidemiol Biomarkers Prev* **22**(11): 2024–2036.
- Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2007) A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev* **16**(11): 2304–2313.
- IARC (1994) IARC working group on the evaluation of carcinogenic risks to humans: some industrial chemicals. Lyon, 15–22 February 1994. *IARC Monogr Eval Carcinog Risks Hum* **60**: 1–560.
- Jamison PM, Noone AM, Ries LA, Lee NC, Edwards BK (2013) Trends in endometrial cancer incidence by race and histology with a correction for the prevalence of hysterectomy, SEER 1992 to 2008. *Cancer Epidemiol Biomarkers Prev* **22**(2): 233–241.
- Johnson KA, Gorzinski SJ, Bodner KM, Campbell RA, Wolf CH, Friedman MA, Mast RW (1986) Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol Appl Pharmacol* **85**(2): 154–168.
- Kaaks R, Lukanova A, Kurzer MS (2002) Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. *Cancer Epidemiol Biomarkers Prev* **11**(12): 1531–1543.
- Larsson SC, Hakansson N, Akeson A, Wolk A (2009) Long-term dietary acrylamide intake and risk of endometrial cancer in a prospective cohort of Swedish women. *Int J Cancer* **124**(5): 1196–1199.
- LoPachin RM, Gavin T (2008) Acrylamide-induced nerve terminal damage: relevance to neurotoxic and neurodegenerative mechanisms. *J Agric Food Chem* **56**(15): 5994–6003.
- Obon-Santacana M, Slimani N, Lujan-Barroso L, Travier N, Hallmans G, Freisling H, Ferrari P, Boutron-Ruault MC, Racine A, Clavel F, Saieva C, Pala V, Tumino R, Mattiello A, Vineis P, Arguelles M, Ardanaz E, Amiano P, Navarro C, Sanchez MJ, Molina ME, Key T, Khaw KT, Wareham N, Peeters PH, Trichopoulou A, Bamia C, Trichopoulos D, Boeing H, Kaaks R, Katzke V, Ye W, Sund M, Ericson U, Wirfalt E, Overvad K, Tjonneland A, Olsen A, Skeie G, Asli LA, Weiderpass E, Riboli E, Bueno-de-Mesquita HB, Duell EJ (2013) Dietary intake of acrylamide and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. *Ann Oncol* **24**(10): 2645–2651.
- Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, Charrondiere UR, Hemon B, Casagrande C, Vignat J, Overvad K, Tjonneland A, Clavel-Chapelon F, Thiebaut A, Wahrendorf J, Boeing H, Trichopoulos D, Trichopoulou A, Vineis P, Palli D, Bueno-de-Mesquita HB, Peeters PH, Lund E, Engeset D, Gonzalez CA, Barricarte A, Berglund G, Hallmans G, Day NE, Key TJ, Kaaks R, Saracci R (2002) European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* **5**(6B): 1113–1124.
- Schoenfeld D (1982) Partial residuals for the proportional hazards regression model. *Biometrika* **69**(1): 239–241.
- Setiawan VW, Yang HP, Pike MC, McCann SE, Yu H, Xiang YB, Wolk A, Wentzensen N, Weiss NS, Webb PM, van den Brandt PA, van de Vijver K, Thompson PJ, Strom BL, Spurdle AB, Soslow RA, Shu XO, Schairer C, Sacerdote C, Rohan TE, Robien K, Risch HA, Ricceri F, Rebbeck TR, Rastogi R, Prescott J, Polidoro S, Park Y, Olson SH, Moysich KB, Miller AB, McCullough ML, Matsuno RK, Magliocco AM, Lurie G, Lu L, Lissowska J, Liang X, Lacey Jr. JV, Kolonel LN, Henderson BE, Hankinson SE, Hakansson N, Goodman MT, Gaudet MM, Garcia-Closas M, Friedenreich CM, Freudenheim JL, Doherty J, De Vivo I, Courneya KS, Cook LS, Chen C, Cerhan JR, Cai H, Brinton LA, Bernstein L, Anderson KE, Anton-Culver H, Schouten LJ, Horn-Ross PL (2013) Type I and II endometrial cancers: have they different risk factors? *J Clin Oncol* **31**(20): 2607–2618.
- Slimani N, Ferrari P, Ocke M, Welch A, Boeing H, Liere M, Pala V, Amiano P, Lagiou A, Mattisson I, Stripp C, Engeset D, Charrondiere R, Buzzard M, Staveren W, Riboli E (2000) Standardization of the 24-hour diet recall calibration method used in the European Prospective Investigation into Cancer and Nutrition (EPIC): general concepts and preliminary results. *Eur J Clin Nutr* **54**(12): 900–917.
- Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M (2002) Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem* **50**(17): 4998–5006.
- Tavassoli FA, Devilee P (2003) Tumours of the Uterine Corpus. In *World Health Organization Classification Tumours. Pathology and Genetics of Tumours of the Breast and Female Genital Organs*, Tavassoli FA, Devilee P (eds) pp 218–258. IARC Press: Lyon.
- Terry PD, Rohan TE, Franceschi S, Weiderpass E (2004) Endometrial Cancer. In *Tobacco and Public Health: Science and Policy*, Boyle P, Gray N, Henningfield J, Seffrin J, Zatonski W (eds) pp 523–545. Oxford University Press: New York.
- Vesper HW, Slimani N, Hallmans G, Tjonneland A, Agudo A, Benetou V, Bingham S, Boeing H, Boutron-Ruault MC, Bueno-de-Mesquita HB, Chirlaque D, Clavel-Chapelon F, Crowe F, Drogan D, Ferrari P, Johansson I, Kaaks R, Linseisen J, Lund E, Manjer J, Mattiello A, Palli D, Peeters PH, Rinaldi S, Skeie G, Trichopoulou A, Vineis P, Wirfalt E, Overvad K, Stromberg U (2008) Cross-sectional study on acrylamide hemoglobin adducts in subpopulations from the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. *J Agric Food Chem* **56**(15): 6046–6053.
- Voss S, Charrondiere UR, Slimani N, Kroke A, Riboli E, Wahrendorf J, Boeing H (1998) [EPIC-SOFT a European computer program for 24-hour dietary protocols]. *Z Ernahrungswiss* **37**(3): 227–233.
- Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, Day NE (2003) Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Public Health Nutr* **6**(4): 407–413.
- Willett WC (1998) *Nutritional Epidemiology*. Oxford University Press: New York.
- Wilson KM, Balter K, Adami HO, Gronberg H, Vikstrom AC, Paulsson B, Tornqvist M, Mucci LA (2009) Acrylamide exposure measured by food frequency questionnaire and hemoglobin adduct levels and prostate cancer risk in the Cancer of the Prostate in Sweden Study. *Int J Cancer* **124**(10): 2384–2390.
- Wilson KM, Mucci LA, Rosner BA, Willett WC (2010) A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancers. *Cancer Epidemiol Biomarkers Prev* **19**(10): 2503–2515.

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Dietary intake of acrylamide and epithelial ovarian cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort

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Abstract

Acrylamide, classified in 1994 by IARC as ‘probably carcinogenic’ to humans, was discovered in 2002 in some heat-treated, carbohydrate-rich foods. The association between dietary acrylamide intake and epithelial ovarian cancer risk (EOC) has been previously studied in one case-control and three prospective cohort studies which obtained inconsistent results, and could not further examine histological subtypes other than serous EOC. The present study was carried out in the European Prospective Investigation into Cancer and Nutrition (EPIC) sub-cohort of women ($n=325,006$). Multivariate Cox proportional hazards models were used to assess the association between questionnaire-based acrylamide intake and EOC risk. Acrylamide was energy-adjusted using the residual method, and was evaluated both as a continuous variable (per 10 μ g/day) and in quintiles; when subgroups by histological EOC subtypes were analyzed, acrylamide intake was evaluated in quartiles. During a mean follow-up of 11 years, 1,191 incident EOC cases were diagnosed. At baseline, the median acrylamide intake in EPIC was 21.3 μ g/day. No associations, and no evidence for a dose-response were observed between energy-adjusted acrylamide intake and EOC risk (HR_{10 μ g/day}:1.02, 95%CI:0.96-1.09; HR_{Q5vsQ1}:0.97, 95%CI:0.76-1.23). No differences were seen when invasive EOC subtypes (582 serous, 118 endometrioid, and 79 mucinous tumors) were analyzed separately. This study did not provide evidence that acrylamide

intake, based on food intake questionnaires, was associated with risk for EOC in EPIC. Additional studies with more reliable estimates of exposure based on biomarkers may be needed.

Keywords

acrylamide; epithelial ovarian cancer; cohort; nutrition; EPIC

INTRODUCTION

Acrylamide has been classified as “probably carcinogenic to humans” by the International Agency for Research on Cancer (IARC; group 2A) since 1994 (1); however public health concern increased when Swedish researchers reported acrylamide in common carbohydrate-rich foods treated at high temperatures (e.g., fried potatoes, potato crisps, bread, and crisp bread) (2). In the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, the major dietary sources of acrylamide (based on a 24-h dietary recall; DR) came from bread, rusks, coffee, potatoes, cakes, biscuits, and cookies(3). An important non-dietary source of exposure is cigarette smoking. It is known that smokers have higher mean circulating acrylamide hemoglobin adducts levels than nonsmokers (4).

Hormone-related tumors and other tumors have been identified in rodents after oral administration of acrylamide (5). In humans, acrylamide is neurotoxic, and it has been hypothesized that it may also have hormonal effects (6); however, acrylamide is thought to play a role in cancer risk by means of its metabolite glycidamide. The conversion of acrylamide to glycidamide (a chemically reactive epoxide and mutagen in animals) is mediated by the Cyp2e1 enzyme system (7).

One case-control study and three prospective cohort studies have evaluated the association between dietary acrylamide intake and epithelial ovarian cancer (EOC), but results were inconsistent. Both the Italian case-control study (8) and the prospective Swedish Mammography Cohort (SMC) (9) study reported null associations, the Nurses’ Health Study (NHS) suggested an increased risk for serous tumors (HR_{Q5vsQ1} : 1.58, 95%CI: 0.99-2.52; P for trend: 0.04), and for serous invasive tumors (HR_{Q5vsQ1} : 1.67, 95%CI: 0.99-2.81; P for trend: 0.04) (10), whereas the Netherlands Cohort Study (NLCS) reported positive associations for overall EOC (HR_{Q5vsQ1} : 1.78, 95%CI: 1.10-2.88; P for trend: 0.02) (11). The NHS included in the analyses both borderline and invasive tumors, whereas in the NLCS and SMC studies all borderline tumors were excluded. The Italian case-control study did not report associations by tumor invasiveness (8-11).

The present study evaluated the association between questionnaire-based intake of acrylamide and the risk of overall EOC. Given that there are risk factor and clinical behavior differences between histological subtypes (12-14), we also evaluated the association between acrylamide intake and serous, endometrioid, and mucinous subtypes and tumor invasiveness. Secondary objectives were to determine whether this association differed by smoking status (with the intention to remove acrylamide exposure due to smoking), oral contraceptive (OC) use (a strong protective factor for EOC risk) (15), and other baseline participant characteristics.

MATERIALS AND METHODS

Study population

The EPIC study enrolled participants between 1992-1998 in 23 centers from 10 European countries. All participants signed an informed consent, and ethical review boards from the IARC and local centers authorized the study. The EPIC methodology has been described in detail by Riboli et al. Participants reported information on lifestyle, reproductive and anthropometric factors at baseline. Dietary intake was also assessed at baseline through validated country-specific dietary questionnaires (DQs) (16).

The EPIC study recruited 521,330 participants, of which 367,903 are women. Women were excluded from the current analyses because they had prevalent cancer other than nonmelanoma skin cancer (n=19,853), had a bilateral oophorectomy (n=10,404), had incomplete follow-up data (n=2,896), had no lifestyle or dietary information (n=3,239), no information on dietary intake of acrylamide at baseline (n=3), or had an extreme ratio of energy intake to energy required (n=6,502); resulting in 325,006 participants for this analysis. Follow-up was estimated until cancer diagnosis (except non-melanoma skin cancer), emigration, death, or until the end of follow-up (centers dates vary from December 2004 to June 2010).

Incident EOC was assessed via population cancer registries, or via a combination of methods (health insurance records, cancer and pathology registries, and active follow-up) (16). Incident EOC included ovarian, fallopian tube, and primary peritoneal cancers, classified according to the International Classification of Diseases 10th revision as: C56.9, C57.0, and C48, respectively.

Overall EOC comprised borderline (n=96; 8%) and invasive tumors (n=1,095; 92%). Invasive EOC were classified as serous (n=582, 53%), not otherwise specified (NOS; n=249, 23%; NOS included adenocarcinomas, carcinomas, and cystadenocarcinoma), endometrioid (n=118, 11%), mucinous (n=79, 7%), clear cell (n=51, 5%), and other tumors (n=16, 1%).

Acrylamide intake assessment

Details of the EPIC acrylamide database have been previously published (17, 18). Briefly, a harmonized acrylamide database was compiled using mean acrylamide levels in foods mainly derived from the EU monitoring database maintained by the European Community Institute for Reference Materials and Measurements (IRMM) (<https://irmm.jrc.ec.europa.eu/activities/acrylamide/Pages/database.aspx>). The DQ items, and when available, their specific description (e.g. 'baked potatoes') were matched with the acrylamide database.

Statistical analysis

Cox proportional hazards models were used to estimate hazards ratios (HR) and 95% confidence intervals (95%CI) for acrylamide intake and EOC risk. Acrylamide intake was energy-adjusted using the residual method (19), and was analyzed both as a continuous variable (10 µg/day; average daily intake in 10 microgram increments) and as quintiles of

intake ($\mu\text{g/day}$) based on the distribution of acrylamide intake in the EPIC sub-cohort of women at baseline. Analyses were also performed by histological subtypes. Due to the number of cases, quartiles of acrylamide intake ($\mu\text{g/day}$) were used to analyze subgroups by histological subtype.

All models had age at the time scale, and were stratified by study center to control for center effects (i.e. questionnaire design and follow-up procedures), and by age at recruitment (1-yr categories).

Multivariable models were adjusted for: body mass index (BMI), smoking status, OC use, baseline menopausal status combined with age at menopause, parity, age at menarche, and energy intake. If needed, missing values were categorized and included as a separate category in the analyses. Additional covariates were evaluated but were not included in models because they did not change the HR by $>10\%$: age at first menstrual period (years), duration of using OC (years), HRT use (yes, no, unknown), duration of using HRT (years), alcohol (non-consumers, consumers), education level (none, primary, technical/professional, secondary, and higher education), physical activity using the Cambridge index (20), waist-to-hip ratio, total fats (g/day), total carbohydrates (g/day), vegetables (g/day), and coffee (ml/day).

Stratified analyses were carried out by smoking status (an important source of acrylamide), OC use (a protective factor for EOC risk), alcohol intake, and BMI (which may both affect the activity of Cyp2e1, important in acrylamide metabolism) (3), and by geographical region (Northern: France, the United Kingdom, The Netherlands, Germany, Sweden, Denmark, and Norway; Southern: Italy, Spain, and Greece). Sensitivity analyses excluding the first 2 years of follow-up were performed with the aim to minimize the influence of preclinical disease on dietary habits.

The median value for each acrylamide quartile or quintile was estimated and included in a score test to evaluate dose-response trends. The proportional hazards assumption (PH), assessed using Schoenfeld residuals (21), was met for all the analyses. All analyses were performed using SAS v. 9.1 (Cary, North Carolina, USA); STATA (College Station, Texas, USA) was used to test the PH assumption.

RESULTS

After a mean follow-up of 11 years, there were 1,191 incident EOC cases. In the present sub-cohort, the median acrylamide intake at baseline was $21.3 \mu\text{g/day}$, and the 25th–75th percentile range was 14.7 – $30.4 \mu\text{g/day}$ (mean and standard deviation acrylamide intake: $23.8 \pm 13.0 \mu\text{g/day}$). The highest median intakes were found in Denmark, the UK, and the Netherlands, whereas Italy and Norway had the lowest median intakes (Table 1). The mean age at diagnosis was 61 years. Description of baseline characteristics of the current cohort of women can be found in Table 2.

No associations were observed between energy-adjusted dietary intake of acrylamide and risk of EOC overall or by histological subtypes (Table 3). Moreover, there was no evidence for linear dose-response trends (Table 3). Results remained unchanged when we excluded

from the analyses those cases diagnosed during the first two years of follow-up (data not shown).

None of the stratified analyses by smoking status (never, ever smokers), or by OC use (never, ever users) showed an association between EOC risk and acrylamide intake. Likewise, no association was observed when subgroups by alcohol intake (never, ever drinkers), BMI (<25 , ≥ 25 kg/m²), or geographical region were evaluated. The same pattern was seen when these associations were analyzed for different histological subtypes (serous, endometrioid, and mucinous tumors). Further, in order to increase statistical power, we also evaluated serous tumors combined with tumors that were not specified (NOS), and endometrioid tumors with clear cell tumors; however, the estimates did not vary.

All models were also evaluated using acrylamide intake without energy-adjustment using the residual method, and results were similar to those presented in table 3 (data not shown).

DISCUSSION

The present study did not find an association between acrylamide intake and EOC risk overall, or in any of the histological subtypes that were evaluated. Relative risks also remained unchanged when subgroups were analyzed.

The relation between dietary acrylamide intake and EOC risk has been previously evaluated in one case-control and three prospective cohort studies. Our results are in agreement with the Italian case-control (8) and SMC studies (9); moreover, average daily acrylamide intakes (23.33 ± 17.65 and 24.6 ± 7.6 µg/day, respectively) in these two studies were similar to the average reported in the current EPIC sub-cohort (23.8 ± 13.0 µg/day). In contrast to our findings, increased relative risks were observed in high acrylamide consumers in two cohort studies: the NLCS for the entire cohort and among never smoking women (11), and the NHS for serous tumors (10). It is noteworthy that compared to the present EPIC sub-cohort, both the NLCS and the NHS had similar acrylamide intake medians in the lowest quintiles (9.5 and 8.7 µg/day, respectively) to EPIC (9.8 µg/day); however, median intakes in the highest quintiles (36.8 and 25.1 µg/day, respectively) were somewhat lower than in EPIC (41.0 µg/day).

Strengths of this study are the prospective cohort design, and the large sample size compared to previous studies which included 1,031 (8), 195 (11), 368 (9), and 416 (10) cases. This enabled us to further investigate specific histological subtypes, such as serous and endometrioid tumors; nevertheless, we were unable to perform exhaustive analyses for clear cell and mucinous tumors. There are other limitations that should be noted. Firstly, the estimation of dietary acrylamide consumption was based on DQs, and the correlation coefficient between DQs and a single 24-h DR in EPIC was low (0.35 and 0.17 for crude and adjusted correlation coefficient, respectively) (22). Additionally, studies that evaluated correlation coefficients between acrylamide intake (based on DQs) and biomarkers of exposure measured as hemoglobin adducts have reported mixed results, with correlation ranging from 0.08 to 0.43, and with most of the studies falling on the lower end of the range, including EPIC (22-27). Thus, we included energy intake in all regression models, since

based on a previous analysis in EPIC, acrylamide intake estimates improved after this adjustment (22). Secondly, misclassification of acrylamide exposure may exist since information on cooking methodology was not available in some EPIC centers. Finally, we acknowledge that measurement error may be present in our dietary acrylamide estimates since a harmonized acrylamide database was used, and because DQs in EPIC were not specifically designed to assess dietary acrylamide exposure; nonetheless to reduce the impact of measurement error, estimates were energy-adjusted using the residual method (19), and all models were stratified by center with the intention to partially account for the variation in dietary patterns across the 10 EPIC countries.

This is the third questionnaire-based study to conclude that acrylamide intake is not associated with risk for EOC. Recently, the NHS conducted the first epidemiologic study that assessed the association between acrylamide measured as hemoglobin adducts and EOC risk, but failed to replicate the positive associations observed when acrylamide intake was based on food frequency questionnaires (28). Additional studies with biomarkers of internal dose with a larger number of cases should be carried out; however, based on our data and the previous inconsistent findings in the literature, acrylamide appears unlikely to play a major role in ovarian cancer carcinogenesis.

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Reference List

- (1). IARC. IARC working group on the evaluation of carcinogenic risks to humans: some industrial chemicals. Lyon, 15-22 February 1994. IARC Monogr Eval Carcinog Risks Hum. 1994; 60:1–560. [PubMed: 7869568]
- (2). Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem. 2002; 50(17):4998–5006. [PubMed: 12166997]
- (3). Freisling H, Moskal A, Ferrari P, et al. Dietary acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition differs greatly according to geographical region. Eur J Nutr. 2013; 52(4):1369–80. [PubMed: 23238529]
- (4). Vesper HW, Bernert JT, Ospina M, et al. Assessment of the relation between biomarkers for smoking and biomarkers for acrylamide exposure in humans. Cancer Epidemiol Biomarkers Prev. 2007; 16(11):2471–8. [PubMed: 18006939]
- (5). Friedman MA, Dulak LH, Stedham MA. A lifetime oncogenicity study in rats with acrylamide. Fundam Appl Toxicol. 1995; 27(1):95–105. [PubMed: 7589934]

- (6). Hogervorst JG, Baars BJ, Schouten LJ, et al. The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. *Crit Rev Toxicol*. 2010; 40(6):485–512. [PubMed: 20170357]
- (7). Doroshyenko O, Fuhr U, Kunz D, et al. In vivo role of cytochrome P450 2E1 and glutathione-S-transferase activity for acrylamide toxicokinetics in humans. *Cancer Epidemiol Biomarkers Prev*. 2009; 18(2):433–43. [PubMed: 19190172]
- (8). Pelucchi C, Galeone C, Levi F, et al. Dietary acrylamide and human cancer. *Int J Cancer*. 2006; 118(2):467–71. [PubMed: 16003724]
- (9). Larsson SC, Akesson A, Wolk A. Long-term dietary acrylamide intake and risk of epithelial ovarian cancer in a prospective cohort of Swedish women. *Cancer Epidemiol Biomarkers Prev*. 2009; 18(3):994–7. [PubMed: 19223560]
- (10). Wilson KM, Mucci LA, Rosner BA, Willett WC. A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancers. *Cancer Epidemiol Biomarkers Prev*. 2010; 19(10):2503–15. [PubMed: 20693310]
- (11). Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2007; 16(11):2304–13. [PubMed: 18006919]
- (12). Kurian AW, Balise RR, McGuire V, Whittemore AS. Histologic types of epithelial ovarian cancer: have they different risk factors? *Gynecol Oncol*. 2005; 96(2):520–30. [PubMed: 15661246]
- (13). Cho KR, Shih I. Ovarian cancer. *Annu Rev Pathol*. 2009; 4:287–313. [PubMed: 18842102]
- (14). Gram IT, Lukanova A, Brill I, et al. Cigarette smoking and risk of histological subtypes of epithelial ovarian cancer in the EPIC cohort study. *Int J Cancer*. 2012; 130(9):2204–10. [PubMed: 21678398]
- (15). Tsilidis KK, Allen NE, Key TJ, et al. Oral contraceptive use and reproductive factors and risk of ovarian cancer in the European Prospective Investigation into Cancer and Nutrition. *Br J Cancer*. 2011; 105(9):1436–42. [PubMed: 21915124]
- (16). Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr*. 2002; 5(6B):1113–24. [PubMed: 12639222]
- (17). Obon-Santacana M, Slimani N, Lujan-Barroso L, et al. Dietary intake of acrylamide and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. *Ann Oncol*. 2013; 24(10):2645–51. [PubMed: 23857962]
- (18). Obon-Santacana M, Kaaks R, Slimani N, et al. Dietary intake of acrylamide and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort. *Br J Cancer*. 2014
- (19). Willett, WC. *Nutritional Epidemiology*. 3rd ed. Oxford University Press; New York: 2013.
- (20). Wareham NJ, Jakes RW, Rennie KL, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Public Health Nutr*. 2003; 6(4):407–13. [PubMed: 12795830]
- (21). Schoenfeld D. Partial residuals for the proportional hazards regression model. *Biometrika*. 1982; 69(1):239–41.
- (22). Ferrari P, Freisling H, Duell EJ, et al. Challenges in estimating the validity of dietary acrylamide measurements. *Eur J Nutr*. 2013; 52(5):1503–12. [PubMed: 23114503]
- (23). Bjellaas T, Olesen PT, Frandsen H, et al. Comparison of estimated dietary intake of acrylamide with hemoglobin adducts of acrylamide and glycidamide. *Toxicol Sci*. 2007; 98(1):110–7. [PubMed: 17449897]
- (24). Wirfalt E, Paulsson B, Tornqvist M, Axmon A, Hagmar L. Associations between estimated acrylamide intakes, and hemoglobin AA adducts in a sample from the Malmo Diet and Cancer cohort. *Eur J Clin Nutr*. 2008; 62(3):314–23. [PubMed: 17356560]
- (25). Kutting B, Uter W, Drexler H. The association between self-reported acrylamide intake and hemoglobin adducts as biomarkers of exposure. *Cancer Causes Control*. 2008; 19(3):273–81. [PubMed: 17985202]

- (26). Wilson KM, Vesper HW, Tocco P, et al. Validation of a food frequency questionnaire measurement of dietary acrylamide intake using hemoglobin adducts of acrylamide and glycidamide. *Cancer Causes Control*. 2009; 20(3):269–78. [PubMed: 18855107]
- (27). Tran NL, Barraj LM, Murphy MM, Bi X. Dietary acrylamide exposure and hemoglobin adducts--National Health and Nutrition Examination Survey (2003-04). *Food Chem Toxicol*. 2010; 48(11):3098–108. [PubMed: 20696196]
- (28). Xie J, Terry KL, Poole EM, et al. Acrylamide hemoglobin adduct levels and ovarian cancer risk: a nested case-control study. *Cancer Epidemiol Biomarkers Prev*. 2013; 22(4):653–60. [PubMed: 23417989]

Table 1
Estimated dietary intake of acrylamide and epithelial ovarian cancer (EOC) cases in the EPIC sub-cohort of women by country

Country	Cohort sample	Person-years	Acrylamide (µg/day)		Acrylamide (µg/kg-body weight/day)	EOC cases N (%)		Invasive EOC cases by histological subtype					
			Median (QR)	Median (QR)		Median (QR)	Median (QR)	Serous N (%)	Mucinous N (%)	Endometrioid N (%)	Clear cell N (%)	NOS N (%)	Others N (%)
France	65,538	680,305	19.2 (14.3-25.2)	17.7 (14.0-21.9)	0.3 (0.2-0.4)	159 (13.4)	15 (19.0)	97 (16.7)	15 (19.0)	14 (11.9)	2 (3.9)	9 (3.6)	6 (37.5)
Italy	29,277	327,642	9.7 (6.5-13.8)	8.6 (5.4-11.8)	0.2 (0.1-0.2)	104 (8.7)	8 (10.1)	56 (9.6)	8 (10.1)	14 (11.9)	3 (5.9)	15 (6.0)	1 (6.3)
Spain	23,508	283,562	18.4 (11.9-26.9)	19.5 (14.1-26.2)	0.3 (0.2-0.4)	68 (5.7)	3 (3.8)	32 (5.5)	3 (3.8)	10 (8.5)	5 (9.8)	6 (2.4)	2 (12.5)
United Kingdom	50,858	567,697	30.6 (22.4-40.9)	31.2 (24.2-39.7)	0.5 (0.3-0.7)	211 (17.7)	11 (13.9)	73 (12.5)	11 (13.9)	15 (12.7)	14 (27.5)	74 (29.7)	4 (25.0)
The Netherlands	26,074	306,436	29.1 (21.3-38.4)	29.7 (23.1-38.0)	0.4 (0.3-0.6)	105 (8.8)	6 (7.6)	55 (9.5)	6 (7.6)	10 (8.5)	4 (7.8)	20 (8.0)	-
Greece	14,376	140,157	17.6 (12.9-23.4)	18.9 (15.3-23.1)	0.3 (0.2-0.3)	37 (3.1)	1 (1.3)	12 (2.1)	1 (1.3)	3 (2.5)	2 (3.9)	17 (6.8)	1 (6.3)
Germany	26,571	264,226	22.4 (16.9-29.6)	23.6 (19.1-29.7)	0.3 (0.2-0.4)	82 (6.9)	7 (8.9)	53 (9.1)	7 (8.9)	8 (6.8)	-	9 (3.6)	-
Sweden	26,375	349,308	20.6 (15.8-26.9)	22.6 (18.7-27.0)	0.3 (0.2-0.4)	137 (11.5)	14 (17.7)	50 (8.6)	14 (17.7)	10 (8.5)	8 (15.7)	53 (21.3)	2 (12.5)
Denmark	27,403	302,433	34.5 (27.5-42.3)	34.7 (28.4-41.5)	0.5 (0.4-0.6)	140 (11.8)	8 (10.1)	76 (13.1)	8 (10.1)	18 (15.3)	8 (15.7)	30 (12.0)	-
Norway	35,026	340,876	17.4 (13.6-21.5)	20.2 (16.8-23.7)	0.3 (0.2-0.3)	148 (12.4)	6 (7.6)	78 (13.4)	6 (7.6)	16 (13.6)	5 (9.8)	16 (6.4)	-
TOTAL	325,006	3,562,642	21.3 (14.7-30.4)	21.9 (16.0-29.8)	0.3 (0.2-0.5)	1,191	79	582	79	118	51	249	16

EPIC, European Prospective Investigation into Cancer and Nutrition; EOC, epithelial ovarian cancer; NOS, not otherwise specified; QR, quartile range (25–75th percentile).

^aEnergy-adjusted using the residual method.

Table 2
Estimated total dietary intake of acrylamide (energy-adjusted using the residual method)
and covariates at baseline used in the analyses: EPIC sub-cohort (325,006 women)

	Energy-adjusted acrylamide intake (µg/day)				
	<14.6	14.7-19.6	19.7-24.4	24.5-32.3	32.4-222.4
Participants	65,001	65,001	65,002	65,001	65,001
EOC cases	221	207	219	280	264
Energy-adjusted acrylamide intake (µg/d)^a	10.8(7.6-13.0)	17.2(16.0-18.4)	21.9(20.7-23.1)	27.7(25.9-29.8)	39.5(35.4- 45.9)
Age at recruitment^a	51.0(45.5-57.1)	50.4(45.3-56.9)	50.2(44.5-56.6)	50.6(43.8-57.5)	51.7(43.5-58.0)
Age at menopause^{a,b}	50.0(47.0-52.0)	50.0(47.0-52.0)	50.0(46.0-52.0)	50.0(46.0-52.0)	50.0(46.0-52.0)
Menopausal status at baseline(%)					
Premenopausal	35.0	34.1	36.1	37.4	36.9
Postmenopausal	45.2	43.8	42.6	44.2	47.5
Perimenopausal	19.8	22.1	21.3	18.5	15.7
Ever use of OCs (%)					
Yes	49.07	55.63	58.11	61.38	64.89
Unknown	0.64	2.42	4.32	3.70	1.71
Parity (%)					
Nulliparous	12.2	11.9	12.6	16.0	19.4
1 child	17.58	14.61	13.65	13.39	13.39
2 children	41.57	39.94	38.64	36.52	36.02
≥3 children	25.35	27.29	26.36	25.03	23.64
Parous but with missing number of full-term pregnancies	0.4	0.9	1.6	3.3	5.3
Unknown	2.9	5.4	7.1	5.7	2.3
Smoking status (%)					
Never	59.9	60.0	55.4	52.3	49.6
Former	19.5	20.9	23.0	24.3	25.4
Current	18.5	15.6	18.9	21.3	23.8
Unknown	2.2	3.4	2.8	2.1	1.2
Cigarettes per day (smokers only)^{a,b}	11.0(6.0-20.0)	10.0(8.0-20.0)	10.0(10.0-20.0)	10.0(10.0-20.0)	15.0(10.0-20.0)
Time since quitting smoking (y)^{a,b,c}	12.5(6.5-20.0)	14.5(7.0-22.0)	14.5(6.5-22.0)	14.5(6.5-22.0)	14.0(6.0-22.5)
BMI (kg/m²)^a	24.3(21.9-27.4)	23.8(21.6-26.8)	24.0(21.8-27.0)	24.1(21.9-27.1)	24.3(22.0-27.3)
Energy (kcal/day)^a	2033.7(1684.4-2444.0)	1803.9(1487.8-2167.6)	1750.3(1441.8-2113.0)	1813.6(1509.0-2172.1)	1966.1(1655.0-2335.1)

EPIC, European Prospective Investigation into Cancer and Nutrition; EOC, epithelial ovarian cancer; BMI, body mass index; and OCs, oral contraceptives

^a Median and quartile range (25-75th percentile)

^b Percent of women missing the following; age at menopause: 66%; number of cigarettes per day: 55%; and time since quitting smoking: 55%

^c Only in former smokers



Table 3
Hazard Ratios (HR) and 95% confidence intervals (95%CI) for estimated dietary intake of acrylamide (energy-adjusted using the residual method) and epithelial ovarian cancer risk (EOC) in EPIC.

Energy-adjusted acrylamide intake (µg/d)							
	10 µg increments	<14.6	Quintiles				Trend test <i>P</i> -value ^{<i>a</i>}
			14.7-19.6	19.7-24.4	24.5-32.3	32.4-222.4	
EOC							
N cases	1,191	221	207	219	280	264	
HR (95%CI) ^{<i>b</i>}	1.02(0.96-1.09)	1.00 (ref)	0.89(0.72-1.11)	0.87(0.70-1.09)	1.08(0.87-1.34)	0.97(0.76-1.23)	0.73
Borderline							
N cases		96	15	19	27	23	12
HR (95%CI) ^{<i>b</i>}	0.90(0.71-1.13)	1.00 (ref)	1.29(0.60-2.76)	1.75(0.83-3.69)	1.55(0.71-3.42)	0.82(0.32-2.08)	0.56
Invasive							
N cases	1,095	206	188	192	257	252	
HR (95%CI) ^{<i>b</i>}	1.03(0.97-1.10)	1.00 (ref)	0.87(0.69-1.08)	0.81(0.64-1.02)	1.04(0.83-1.31)	0.97(0.75-1.24)	0.60
Serous							
N cases	582	124	103	102	132	121	
HR (95%CI) ^{<i>b</i>}	0.98(0.89-1.07)	1.00 (ref)	0.78(0.59-1.05)	0.72(0.53-0.98)	0.94(0.69-1.28)	0.84(0.60-1.17)	0.72
Not otherwise specified							
N cases	249	28	45	38	64	74	
HR (95%CI) ^{<i>b</i>}	1.09(0.97-1.23)	1.00 (ref)	1.44(0.83-2.50)	1.10(0.61-1.96)	1.54(0.88-2.69)	1.63(0.92-2.90)	0.11
Serous combined with Not otherwise specified							
N cases	831	152	148	140	196	195	
HR (95%CI) ^{<i>b</i>}	1.02(0.95-1.10)	1.00 (ref)	0.90(0.70-1.17)	0.79(0.60-1.03)	1.05(0.81-1.37)	1.00(0.75-1.33)	0.52
Invasive	Endometrioid						
	N cases	118	27	20	19	29	23
	HR (95%CI) ^{<i>b</i>}	1.12(0.93-1.36)	1.00 (ref)	0.77(0.40-1.49)	0.67(0.33-1.34)	1.01(0.51-1.98)	0.72(0.34-1.55)
	Clear Cell						
	N cases	51	6	8	13	12	12
	HR (95%CI) ^{<i>b</i>}	0.92(0.69-1.23)	1.00 (ref)	1.42(0.42-4.73)	1.77(0.54-5.80)	1.42(0.41-4.91)	1.03(0.29-3.74)
	Endometrioid combined with Clear Cell						
	N cases	169	33	28	32	41	35
HR (95%CI) ^{<i>b</i>}	1.05(0.89-1.23)	1.00 (ref)	0.89(0.50-1.57)	0.88(0.49-1.58)	1.07(0.59-1.92)	0.76(0.40-1.45)	
Mucinous							

Energy-adjusted acrylamide intake (µg/d)							
	10 µg increments	Quintiles					Trend test <i>P</i> -value ^a
		<14.6	14.7-19.6	19.7-24.4	24.5-32.3	32.4-222.4	
N cases	79	16	11	13	19	20	
HR (95%CI) ^b	1.17(0.95-1.44)	1.00 (ref)	0.68(0.29-1.60)	0.69(0.29-1.65)	1.11(0.48-2.55)	1.33(0.54-3.28)	0.21

EPIC, European Prospective Investigation into Cancer and Nutrition; EOC, epithelial ovarian cancer.

^a All *p*-values for trend are based on the quintile medians

^b Stratified by age at recruitment and center. Adjusted for total energy intake (1,000 kcal/day), BMI (kg/m²), smoking status (never smokers, current pipe or cigar or occasional smokers, current cigarette smokers: 1-15, 16-25, or ≥26 cigarettes/day, former cigarette smokers who quit >20 years, 11-20 years, or ≤10 years before recruitment), OC use (never, ever, unknown), menopause status combined with age at menopause (premenopausal, peri-menopausal, postmenopausal with: <45, 45-49, 50-52, 53-55, ≥56 years, postmenopausal women with missing age at menopause), and parity (nulliparous, 1, 2, ≥3, parous but with missing number of full-term pregnancies, unknown).

Short Report

Acrylamide and glycidamide hemoglobin adduct levels and endometrial cancer risk: A nested case-control study in nonsmoking postmenopausal women from the EPIC cohort

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Key words: hemoglobin adduct, acrylamide, glycidamide, endometrial cancer, EPIC

Abbreviations: 24hDR: 24-h dietary recall; BMI: body mass index (kg m^{-2}); CI: confidence interval; DQ: dietary questionnaire; EC: endometrial cancer; EPIC: European prospective investigation into cancer and nutrition; FFQ: food frequency questionnaire; HbAA: hemoglobin adducts of acrylamide; HbAA+HbGA: sum of hemoglobin adducts of acrylamide and glycidamide; HbGA: hemoglobin adducts of glycidamide; HbGA/HbAA: ratio of hemoglobin adducts of glycidamide and acrylamide; HPLC/MS/MS: high-performance liquid chromatography–tandem mass spectrometry; HRT: hormone replacement therapy; IARC: international agency for research on cancer; ICC: intraclass correlation coefficient; LOD: limits of detection; LRT: likelihood ratio test; NHS: nurses' health study; OC: oral contraceptive; OR: odds ratio; SHS: second-hand smoke

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Acrylamide, classified in 1994 by IARC as “probably carcinogenic to humans,” was discovered in 2002 in some heat-treated, carbohydrate-rich foods. Four prospective studies have evaluated the association between dietary acrylamide intake and endometrial cancer (EC) risk with inconsistent results. The purpose of this nested case-control study, based on the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, was to evaluate, for the first time, the association between hemoglobin adducts of acrylamide (HbAA) and glycidamide (HbGA) and the risk of developing EC in non-smoking postmenopausal women. Hemoglobin adducts were measured in red blood cells by HPLC/MS/MS. Four exposure variables were evaluated: HbAA, HbGA, their sum (HbAA+HbGA), and their ratio (HbGA/HbAA). The association between hemoglobin adducts and EC was evaluated using unconditional multivariable logistic regression models, and included 383 EC cases (171 were type-I EC), and 385 controls. Exposure variables were analyzed in quintiles based on control distributions. None of the biomarker variables had an effect on overall EC ($HR_{HbAA;Q5vsQ1}$: 0.84, 95%CI: 0.49–1.48; $HR_{HbGA;Q5vsQ1}$: 0.94, 95%CI: 0.54–1.63) or type-I EC risk. Additionally, none of the subgroups investigated (BMI < 25 vs. ≥ 25 kg m⁻², alcohol drinkers vs. never drinkers, oral contraceptive users vs. non-users) demonstrated effect measure modification. Hemoglobin adducts of acrylamide or glycidamide were not associated with EC or type-I EC risk in 768 nonsmoking postmenopausal women from the EPIC cohort.

What’s new?

Acrylamide in food may not lead to endometrial cancer, according to a new report. The carcinogen has provoked public concerns because it can be detected in certain foods. Prospective studies on the relationship between endometrial cancer and dietary acrylamide, however, have produced conflicting results. Taking a different tack, these authors conducted a case-control study, drawing on data from the European Prospective Investigation into Cancer and Nutrition (EPIC). They measured the amounts of certain compounds formed by hemoglobin with acrylamide or glycidamide in nonsmoking, postmenopausal women. Neither of these levels, they report, had any impact on endometrial cancer risk.

The International Agency for Research on Cancer (IARC) classified acrylamide as “probably carcinogenic to humans (group 2A)” based on evidence from animal and *in vitro* studies¹; however scientific interest did not increase until 2002, when Swedish researchers reported acrylamide concentrations in commonly consumed foods.² The principal pathway by which acrylamide is formed in foods is through the Maillard reaction during food processing at temperatures higher than $>120^{\circ}\text{C}$ (*i.e.*, frying or baking),^{2,3} but acrylamide has also been observed in foods treated at lower temperatures (*e.g.*, low moisture drying).⁴ In the European Prospective Investigation into Cancer and Nutrition (EPIC), the major food contributors to dietary acrylamide intake (based on a 24-hr dietary recall; 24hDR) were bread, crisp bread, rusks, coffee and potatoes.⁵

In the human body, acrylamide is conjugated with reduced glutathione for elimination, or is metabolized to glycidamide through the Cyp2e1 enzyme system. In animal studies, after acrylamide administration, both hormone- and nonhormone-related tumors have been observed.¹ Glycidamide is believed to have mutagenic and genotoxic effects in animals, whereas acrylamide is thought to be neurotoxic both in animals and in humans,^{3,6} and may also disrupt hormonal homeostasis.^{7,8}

Acrylamide and its metabolite glycidamide can form adducts with hemoglobin (HbAA and HbGA, respectively), which are stable over the lifespan of erythrocytes (~ 120 days), and thus, have been extensively used as biomarkers of human internal exposure.^{3,9} The mean hemoglobin adduct levels in smokers are at least three to four times higher than non-smokers,¹⁰ and cigarette smoke is considered as one of the major sources of acrylamide exposure. Thus, to assess the impact of dietary acrylamide on health, nonsmokers are considered a more suitable population than smokers.

Cancer of the corpus uteri is the fourth most common incident cancer in European and North American women. The most common type of corpus uteri cancer is endometrial cancer (EC). The 5-year survival rate of EC is high, ranging from 65 to 85%.¹¹ EC has been classified into type-I and type-II tumors; type-I EC is mostly endometrioid adenocarcinoma, and is characterized as an estrogen-dependent tumor. In contrast, type-II EC is usually serous carcinoma, is thought to be estrogen-independent, usually diagnosed in elderly women, and generally has an unfavorable prognosis.^{12,13} Epidemiological data suggest that obesity, diabetes, low physical activity, long-term exposure to estrogens and a history of polycystic ovary syndrome are risk factors for developing EC, and type-I EC in particular.¹⁴ Combined oral contraceptive (OC) use, and tobacco smoking are consistently associated with lower risk of EC.¹⁴ Further, a recent EPIC study observed an inverse association between coffee consumption and EC risk.¹⁵

To date, four prospective epidemiologic studies, including one from EPIC, have evaluated the association between dietary intake of acrylamide (assessed through dietary questionnaires; DQs) and EC risk.^{16–19} Two subsequent meta-analyses concluded that dietary acrylamide intake was not associated with overall EC risk, but increased risk was observed with

higher acrylamide intakes in women who were never smokers at baseline.^{20,21} To our knowledge, this is the first nested-case control study within a prospective cohort study designed to assess the relation between circulating, red blood cell hemoglobin adducts of acrylamide and glycidamide and overall and type-I EC risk.

Material and Methods

The EPIC study comprises 10 European countries and 23 research centers with the aim to evaluate the association between nutrition and lifestyle factors, cancer and other chronic diseases.²² The current study includes participants from 8 of the 10 EPIC countries: Denmark, Norway and one center from Sweden (Malmö) did not participate. For each EPIC center, subjects were followed until cancer diagnosis (except non-melanoma skin cancer), emigration, death or end of follow-up, which varied from December 2005 to June 2010).

The EPIC methodology has been published elsewhere.²² Recruitment began between 1992 and 1998, and participants reported information on dietary habits (referring to the 12 months before recruitment) assessed through country-specific, validated dietary questionnaires (DQs). Additionally, information on tobacco smoking, education, physical activity, anthropometric measures and reproductive factors was also obtained at recruitment. Blood samples were collected at recruitment for $\sim 80\%$ of the EPIC cohort (385,747 of over 500,000 participants). Samples that were stored at the IARC bio-bank were kept in liquid nitrogen (-196°C); whereas blood samples from Umeå were stored at local repositories in freezers (-80°C). The study was approved by the IARC ethical review boards and/or all local ethics committees.

Blood samples were sent to the Centers for Disease Control and Prevention (CDC) Protein Biomarker Laboratory to measure HbAA and HbGA. Details of the methodology have been previously described.¹⁰ Briefly, adduct levels were measured in 300 μL of hemolysed erythrocytes and analyzed by high-performance liquid chromatography–tandem mass spectrometry (HPLC/MS/MS) in a randomized manner. Additionally, for each sample two independent measurements were performed, and results were reported in pmol per g of Hb. The detection limits (LOD) for this method were 3 and 4 pmol g^{-1} Hb for HbAA and HbGA, respectively.

Identification of EC cases was achieved by means of population cancer registries, or through a combination of methods: health insurance records, cancer and pathology registries and active follow-up. EC cases were classified as C54 according to the International Classification of Diseases, 10th revision.

The selection of the study population for the present nested case-control study was based on the algorithm that has been previously published by Cust *et al.* and Peeters *et al.*²³: for each EC case two corresponding controls were randomly selected at the date of diagnosis (subjects free of cancer, with the exception of non-melanoma skin cancer). Cases and controls were matched by study center, menopausal status, age at recruitment (± 6 months), date at blood collection (± 1 month), time of the

Table 1. Description of the study population from a nested case-control study of acrylamide biomarkers and EC in the EPIC cohort

	All EC cases <i>n</i> = 383	Type-I cases <i>n</i> = 171	Controls <i>n</i> = 385
HbAA (pmol/g Hb) ¹	39.9 (31.4–52.4)	40.1 (31.4–52.8)	39.4 (32.1–51.1)
HbGA (pmol/g Hb) ¹	34.1 (25.7–44.6)	33 (25.3–46.2)	33.3 (24.6–43.8)
HbAA+HbGA (pmol/g Hb) ¹	74.4 (57.5–97.6)	72.5 (56.8–97.8)	72.8 (57.2–94.5)
HbGA/HbAA (pmol/g Hb) ¹	0.9 (0.7–1.0)	0.8 (0.7–1.0)	0.8 (0.7–1.0)
Age at recruitment (y) ¹	58.0 (53.5–61.4)	57.7 (53.6–61.0)	58.5 (54.3–61.7)
Age at menopause (y) ¹	49.5 (49.5–52.0)	49.5 (49.5–52.0)	49.5 (49.0–52.0)
BMI (kg m ⁻²) ¹	27.4 (24.1–31.6)	27.4 (24.4–33.2)	26.1 (23.2–29.3)
Country²			
France	33 (8.6)	17 (9.9)	35 (9.1)
Italy	69 (18.0)	24 (14.0)	74 (19.2)
Spain	55 (14.4)	25 (14.6)	72(18.7)
United Kingdom	70 (18.3)	30 (17.5)	60 (15.6)
The Netherlands	56 (14.6)	32 (18.7)	38 (9.9)
Greece	13 (3.4)	3 (1.8)	16 (4.2)
Germany	51 (13.3)	40 (23.4)	56 (14.6)
Sweden	36 (9.4)	0 (0.0)	34(8.8)
Fasting status²			
Unknown	1 (0.3)	1 (0.6)	0 (0.0)
<3 hr	150 (39.2)	77 (45.0)	129 (33.5)
3–6 hr	60 (15.7)	34 (19.9)	64 (16.6)
>6 hr	172 (44.9)	59 (34.5)	192 (49.9)
Alcohol consumption²			
Non drinker	94 (24.5)	37 (21.6)	93 (24.2)
>0–6 g day ⁻¹	168 (43.9)	72 (42.1)	166 (43.1)
>6–12 g day ⁻¹	63 (16.5)	32 (18.7)	67 (17.4)
>12–24 g day ⁻¹	33 (8.6)	19 (11.1)	38 (9.9)
>24–60 g day ⁻¹	25 (6.5)	11 (6.4)	21 (5.5)
Ever use of OC²			
Unknown	10 (2.6)	1 (0.6)	8 (2.1)
No	249 (65.0)	102 (59.7)	237 (61.6)
Yes	124 (32.4)	68 (39.8)	140 (36.4)
Ever use of HRT ²			
Unknown	16 (4.2)	5 (2.9)	15 (3.9)
No	263 (68.7)	114 (66.7)	287 (74.6)
Yes	104 (27.2)	52 (30.4)	83 (21.6)
Parity²			
Unknown	61 (4.4)	31 (2.3)	59 (2.3)
1 child	130 (15.9)	62 (18.1)	140 (15.3)
2 children	105 (33.9)	46 (36.3)	131 (36.4)
≥3 children	62 (27.4)	21 (26.9)	42 (34.0)
Nulliparous	8 (16.2)	7 (12.3)	4 (10.9)
Parous but with missing number of full-term pregnancies	17 (2.1)	4 (4.1)	9 (1.0)

¹Median and quartile range (25th – 75th percentile). ²Number (*n*) and percent (%).

Abbreviations: EC, endometrial cancer; EPIC, European prospective investigation into cancer and nutrition; HbAA, hemoglobin adducts of acrylamide; HbGA, hemoglobin adducts of glycidamide, BMI, body mass index; OC, oral contraceptive; HRT, hormone replacement therapy.

Table 2. OR and 95% CI for biomarkers of acrylamide exposure and EC risk in a nested case-control study in the EPIC cohort

Exposure cut points		Overall EC				Type 1 EC			
		Cases <i>n</i> = 383	Controls <i>n</i> = 385	OR (95%CI)	<i>p</i> - trend	Cases <i>n</i> = 171	Controls <i>n</i> = 385	OR (95%CI)	<i>p</i> - trend
HbAA	≤29.4	77	74	1.00 (ref)	0.94	33	74	1.00 (ref)	0.94
	29.5–36.1	75	80	0.82 (0.49–1.37)		33	80	0.94 (0.49–1.84)	
	36.2–43.6	74	77	0.96 (0.57–1.61)		36	77	1.21 (0.62–2.36)	
	43.7–54.3	73	77	0.87 (0.51–1.48)		30	77	0.96 (0.49–1.92)	
	>54.3	84	77	0.85 (0.49–1.46)		39	77	0.96 (0.48–1.92)	
	Continuous			1.00 (0.99–1.01)				1.00 (0.99–1.02)	
	Continuous-Log ₂			1.00 (0.68–1.47)				1.03 (0.62–1.70)	
HbGA	≤23	56	76	1.00 (ref)	0.74	29	76	1.00 (ref)	0.92
	23.1–29.4	85	78	1.28 (0.76–2.15)		42	78	1.31 (0.68–2.52)	
	29.5–37.6	87	77	1.20 (0.71–2.04)		30	77	1.01 (0.51–2.01)	
	37.7–46.9	75	77	1.06 (0.62–1.83)		29	77	1.03 (0.52–2.06)	
	>46.9	80	77	0.94 (0.54–1.63)		41	77	1.06 (0.53–2.12)	
	Continuous			1.00 (0.98–1.01)				1.00 (0.99–1.01)	
	Continuous-Log ₂			0.92 (0.66–1.28)				1.00 (0.66–1.50)	
Sum of HbAA + HbGA	≤53.6	67	77	1.00 (ref)	0.95	34	77	1.00 (ref)	0.97
	53.7–66.3	81	76	1.16 (0.69–1.96)		38	76	1.15 (0.59–2.23)	
	66.4–81.8	78	78	0.99 (0.59–1.67)		30	78	0.91 (0.47–1.78)	
	81.9–100.2	73	77	1.05 (0.61–1.81)		29	77	0.98 (0.49–1.96)	
	>100.2	84	77	0.95 (0.55–1.63)		40	77	0.97 (0.49–1.91)	
	Continuous			1.00 (0.99–1.01)				1.00 (0.99–1.01)	
	Continuous-Log ₂			0.97 (0.67–1.41)				1.02 (0.64–1.63)	
Ratio of HbGA/HbAA	≤0.69	62	76	1.00 (ref)	0.16	27	76	1.00 (ref)	0.02
	0.70–0.80	92	78	1.29 (0.78–2.14)		49	78	1.93 (1.01–3.69)	
	0.81–0.88	57	73	0.72 (0.42–1.26)		24	73	0.75 (0.36–1.56)	
	0.89–0.98	73	78	0.79 (0.46–1.35)		29	78	0.81 (0.39–1.68)	
	>0.98	99	80	1.08 (0.64–1.84)		42	80	1.45 (0.73–2.88)	
	Continuous			0.82 (0.26–2.54)				0.99 (0.19–5.05)	

All models are adjusted for age at recruitment, country, fasting status, date at blood collection, time of the day of blood collection, OC use, HRT use, alcohol intake, parity, age at menopause, and BMI.

Abbreviations: OR, odds ratio; CI, confidence interval; EC, endometrial cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; HbAA, hemoglobin adducts of acrylamide; HbGA, hemoglobin adducts of glycidamide.

day of blood draw (± 1 hrs), and fasting status (<3 , $3-6$, >6 hrs). Individual matching was broken in the present study (one control per case) because we only included women who were non-smokers, defined as women who reported never smoking or who quit smoking ≥ 5 years before recruitment, and who were postmenopausal at blood draw, defined as women who reported not having menses ≥ 1 year before recruitment.

A total of 771 subjects (385 EC cases and 386 controls) were included in the study. Of these, three had to be excluded due to the lack of information on HbAA ($n = 2$ cases) or HbGA ($n = 1$ control), leaving 383 EC cases and 385 controls included in the final analyses. Only one observation had an HbGA value below the LOD; thus, we assigned half of the corresponding value of the LOD ($2 \text{ pmol g}^{-1} \text{ Hb}$). Tumor histology was avail-

able for 372 (97%) cases, of which 171 (46%) were classified as endometrioid tumors (type-I), 14 (4%) as serous/clear cell tumors (type-II), and 187 (50%) as other types. "Overall EC" comprises type-I, type-II, and tumors that were classified as others or undefined for histology.

To improve normality of the distributions, all biomarker variables were log-transformed (\log_2) and were evaluated as: $\log_2 \text{HbAA}$, $\log_2 \text{HbGA}$, sum of total adducts [$\log_2 (\text{HbAA} + \text{HbGA})$], and HbGA/HbAA ratio. Additionally, these four continuous variables were categorized into quintiles based on the distribution in the control group. Unconditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CI). Analyses were also performed separately for type-I EC tumors.

Table 3. Stratified analyses: OR and 95% CI for biomarkers of acrylamide exposure and EC risk in a nested case-control study in the EPIC cohort.

	<25 kg m ⁻²						≥25 kg m ⁻²						Never drinkers						Drinkers						Nonoral contraceptive users						Oral contraceptive users					
	Cutpoints		Cases		OR (95%CI) ¹		Cases		Controls		OR (95%CI) ¹		Cases		Controls		OR (95%CI) ²		Cases		Controls		OR (95%CI) ²		Cases		Controls		OR (95%CI) ³		Cases		Controls		OR (95%CI) ³	
HbAA	≤30.3	18	21	1.00 (ref)	59	53	1.00 (ref)	21	20	1.00 (ref)	56	54	1.00 (ref)	56	54	1.00 (ref)	56	54	1.00 (ref)	56	54	1.00 (ref)	56	54	1.00 (ref)	56	54	1.00 (ref)	19	18	1.00 (ref)	19	18	1.00 (ref)		
	30.4–37.6	25	32	0.79 (0.29–2.15)	50	48	0.91 (0.49–1.71)	21	18	1.32 (0.47–3.69)	54	62	0.71 (0.39–1.31)	54	62	0.71 (0.39–1.31)	54	62	0.71 (0.39–1.31)	54	62	0.71 (0.39–1.31)	54	62	0.71 (0.39–1.31)	54	62	0.71 (0.39–1.31)	29	30	0.87 (0.47–1.63)	29	30	0.87 (0.47–1.63)		
	37.7–45.3	18	40	0.47 (0.17–1.35)	56	37	1.37 (0.72–2.60)	14	22	0.66 (0.22–1.97)	60	55	1.06 (0.58–1.95)	60	55	1.06 (0.58–1.95)	60	55	1.06 (0.58–1.95)	60	55	1.06 (0.58–1.95)	60	55	1.06 (0.58–1.95)	60	55	1.06 (0.58–1.95)	25	30	0.98 (0.52–1.84)	25	30	0.98 (0.52–1.84)		
	45.4–56.0	27	27	1.03 (0.35–3.01)	46	50	0.60 (0.31–1.16)	16	20	0.98 (0.31–3.03)	57	57	0.88 (0.47–1.66)	57	57	0.88 (0.47–1.66)	57	57	0.88 (0.47–1.66)	57	57	0.88 (0.47–1.66)	57	57	0.88 (0.47–1.66)	57	57	0.88 (0.47–1.66)	26	31	0.92 (0.47–1.79)	26	31	0.92 (0.47–1.79)		
	>56.0	30	34	0.84 (0.29–2.41)	54	43	0.87 (0.44–1.70)	22	13	1.24 (0.36–4.28)	62	64	0.75 (0.40–1.41)	62	64	0.75 (0.40–1.41)	62	64	0.75 (0.40–1.41)	62	64	0.75 (0.40–1.41)	62	64	0.75 (0.40–1.41)	62	64	0.75 (0.40–1.41)	25	31	1.13 (0.58–2.20)	25	31	1.13 (0.58–2.20)		
LRT ⁴			0.06									0.42																		0.63						
HbGA	≤23	21	26	1.00 (ref)	35	50	1.00 (ref)	7	15	1.00 (ref)	49	61	1.00 (ref)	49	61	1.00 (ref)	49	61	1.00 (ref)	49	61	1.00 (ref)	49	61	1.00 (ref)	49	61	1.00 (ref)	16	27	1.00 (ref)	16	27	1.00 (ref)		
	23.1–29.4	26	37	0.75 (0.30–1.87)	59	41	1.88 (0.98–3.62)	29	18	3.89 (1.11–13.71)	56	60	0.97 (0.54–1.75)	56	60	0.97 (0.54–1.75)	56	60	0.97 (0.54–1.75)	56	60	0.97 (0.54–1.75)	56	60	0.97 (0.54–1.75)	56	60	0.97 (0.54–1.75)	35	24	0.93 (0.49–1.77)	35	24	0.93 (0.49–1.77)		
	29.5–37.6	31	36	0.60 (0.24–1.54)	56	41	1.73 (0.87–3.44)	14	22	1.13 (0.30–4.18)	73	55	1.33 (0.74–2.40)	73	55	1.33 (0.74–2.40)	73	55	1.33 (0.74–2.40)	73	55	1.33 (0.74–2.40)	73	55	1.33 (0.74–2.40)	73	55	1.33 (0.74–2.40)	27	33	1.36 (0.70–2.64)	27	33	1.36 (0.70–2.64)		
	37.7–46.9	19	27	0.54 (0.19–1.59)	56	50	1.38 (0.71–2.69)	19	23	1.62 (0.44–6.00)	56	54	1.02 (0.55–1.89)	56	54	1.02 (0.55–1.89)	56	54	1.02 (0.55–1.89)	56	54	1.02 (0.55–1.89)	56	54	1.02 (0.55–1.89)	56	54	1.02 (0.55–1.89)	23	27	1.00 (0.51–1.98)	23	27	1.00 (0.51–1.98)		
	>46.9	21	28	0.55 (0.19–1.59)	59	49	1.25 (0.63–2.49)	25	15	2.17 (0.54–8.79)	55	62	0.76 (0.41–1.42)	55	62	0.76 (0.41–1.42)	55	62	0.76 (0.41–1.42)	55	62	0.76 (0.41–1.42)	55	62	0.76 (0.41–1.42)	55	62	0.76 (0.41–1.42)	23	29	1.16 (0.58–2.30)	23	29	1.16 (0.58–2.30)		
LRT ⁴			0.35									0.05																		0.07						
Sum of HbAA + HbGA																																				
≤53.6			20		24		1.00 (ref)		47		53		1.00 (ref)		53		60		1.00 (ref)		49		55		1.00 (ref)		18		22		1.00 (ref)					
53.7–66.3			24		33				57		43		26		19				55		57		46		45		33		28							

Table 3. Stratified analyses: OR and 95% CI for biomarkers of acrylamide exposure and EC risk in a nested case-control study in the EPIC cohort. (Continued)

Cutpoints	<25 kg m ⁻²				≥25 kg m ⁻²				Never drinkers				Drinkers				Nonoral contraceptive users				Oral contraceptive users			
	Cases	Controls	OR (95%CI) ¹		Cases	Controls	OR (95%CI) ¹		Cases	Controls	OR (95%CI) ²		Cases	Controls	OR (95%CI) ²		Cases	Controls	OR (95%CI) ³		Cases	Controls	OR (95%CI) ³	
66.4–81.8	24	41	0.91 (0.34–2.46)		54	37	1.41 (0.75–2.65)		12	21	2.17 (0.69–6.76)		66	57	0.93 (0.50–1.71)		51	45	1.19 (0.63–2.27)		24	31	1.30 (0.47–3.54)	
81.9–100.2	26	25	1.04 (0.36–2.98)		47	52	0.86 (0.44–1.67)		17	23	1.07 (0.32–3.55)		56	54	1.09 (0.59–2.03)		45	44	1.21 (0.62–2.37)		25	31	0.89 (0.32–2.50)	
>100.2	24	31	0.76 (0.27–2.20)		60	46	1.09 (0.56–2.12)		25	13	1.64 (0.45–6.00)		59	64	0.80 (0.43–1.48)		58	48	1.25 (0.64–2.42)		24	28	0.65 (0.22–1.89)	
LRT ⁴			0.09								0.14								0.68					
Ratio of HbGA/HbAA	34	40	1.00 (ref)		28	36	1.00 (ref)		8	13	1.00 (ref)		54	63	1.00 (ref)		38	37	1.00 (ref)		23	37	1.00 (ref)	
0.70–0.80	35	39	1.14 (0.51–2.53)		57	39	1.63 (0.79–3.35)		24	13	2.79 (0.75–10.34)		68	65	1.08 (0.62–1.88)		56	48	1.05 (0.54–2.06)		35	28	2.47 (1.03–5.94)	
0.81–0.88	14	30	0.45 (0.17–1.16)		43	43	1.10 (0.52–2.30)		8	18	0.44 (0.10–1.86)		49	55	0.81 (0.44–1.48)		35	42	0.63 (0.31–1.31)		20	29	1.10 (0.42–2.87)	
0.89–0.98	16	22	0.63 (0.22–1.79)		57	56	1.03 (0.51–2.07)		18	25	0.69 (0.19–2.48)		55	53	0.83 (0.45–1.52)		51	56	0.73 (0.37–1.46)		21	22	1.17 (0.44–3.11)	
>0.98	19	23	0.75 (0.27–2.07)		80	57	1.59 (0.80–3.17)		36	24	1.12 (0.33–3.82)		63	56	1.01 (0.55–1.86)		69	54	1.06 (0.53–2.10)		25	24	1.25 (0.47–3.37)	
LRT ⁴			0.76								0.16								0.56					

¹Adjusted for age at recruitment, country, fasting status, date at blood collection, OC use, HRT use, alcohol intake, parity, and age at menopause. ²Adjusted for age at recruitment, country, fasting status, date at blood collection, OC use, HRT use, parity, age at menopause, and BMI. ³Adjusted for age at recruitment, country, fasting status, date at blood collection, HRT use, alcohol intake, parity, age at menopause, and BMI. ⁴All LRT *p* values for effect measure modification are based on the categorical exposure adduct variable.

Abbreviations: OR, odds ratio; CI, confidence interval; EC, endometrial cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; HbAA, hemoglobin adducts of acrylamide; HbGA, hemoglobin adducts of glycidamide.

All statistical models were adjusted for matching variables (age at recruitment (years), country, date of blood draw, time of day of the blood draw, and fasting status), and other covariates such as ever use of OC (never, ever), ever use of hormone replacement therapy (never, ever; HRT), parity (nulliparous, 1, 2, ≥ 3 , parous but with missing number of full-term pregnancies), age at menopause (years), body mass index (kg m^{-2} ; BMI), and alcohol intake (non-drinkers, drinkers of 0–6, >6–12, >12–24, and >24 g day^{-1}). The following variables were evaluated as potential confounders but were not included in final models because they did not change the risk estimates by >10%: dietary variables (such as coffee, potatoes, biscuits, crackers and cakes), history of diabetes (yes, no), age at menarche (<12, 12, 13, 14, ≥ 15 years), height (cm), weight (kg), hip circumference (cm), waist circumference (cm), physical activity using the Cambridge index²⁴ and education level (none, primary, technical/professional, secondary and higher education).

Effect-measure modification was evaluated for established risk factors, and for factors considered to affect the activity of Cyp2e1: BMI (<25 vs. $\geq 25 \text{ kg m}^{-2}$), HRT use (never vs. ever users), OC (never vs. ever users), and alcohol intake (never vs. ever drinkers)⁵ using a likelihood ratio test (LRT) based on categorical biomarker variables. For each biomarker quartile, the median was estimated, and was included in a score test to evaluate dose-response trends.

The reproducibility of the hemoglobin adducts measurements was assessed using 43 (5%) duplicate blood samples revealing intraclass correlation coefficients of 0.92 for HbAA and 0.95 for HbGA. All statistical tests were two-sided and statistical significance was set at $p < 0.05$. All analyses were performed using SAS v. 9.1 (Cary, NC).

Results

A large number of cases and controls were from Italy and the United Kingdom, and the major proportion of type-I EC cases were from Germany and The Netherlands (Table 1). The median interval between the dates at blood collection and diagnosis was 6.2 years. Among cases, the median (25th–75th percentile) HbAA and HbGA adducts levels were 39.9 (31.4–52.4) and 34.1 (25.7–44.6) pmol/g Hb, respectively; and in controls 39.4 (32.1–51.1) and 33.3 (24.6–43.8) pmol/g Hb, respectively. As compared with controls, cases were slightly younger, had a slightly higher proportion of heavy drinking (6.5% vs. 5.5%), tended to use less OCs (32.4% vs. 36.4%) and more HRT (27.2% vs. 21.6), had higher median BMI values (27.4 vs. 26.1 kg m^{-2}), and were more likely to be nulliparous (16.2% vs. 10.9%). Cases and controls had similar ages at menopause.

No associations and no evidence for linear dose-response trends were observed between biomarkers of dietary acrylamide exposure and overall EC (highest vs. lowest quintiles: $\text{HR}_{\text{HbAA}, \text{Q5vsQ1}}: 0.85$, 95%CI: 0.49–1.46; $\text{HR}_{\text{HbGA}, \text{Q5vsQ1}}: 0.94$, 95%CI: 0.54–1.63) (Table 2). We also restricted the analyses to known type-I EC cases and no statistically significant associations were observed (Table 2). Associations between biomarkers of exposure and overall or type-I EC risk were also

assessed using tertiles, quartiles, and deciles (based on the exposure distribution in the control group), and no significant variations in risk were observed across categories (data not shown).

Subgroup analyses for overall EC were stratified by BMI (<25, $\geq 25 \text{ kg m}^{-2}$), alcohol intake (never drinkers, ever drinkers), HRT use (never HRT users, ever HRT users; data not shown) and OC use (never OC users, ever OC users). No evidence for effect measure modification was observed in any of the subgroups evaluated (all LRT p values >0.05) (Table 3). Because of the small sample size, stratified analyses for type-I EC were conducted using tertiles, and results indicated no heterogeneity (data not shown).

Discussion

The present nested case-control study within the EPIC cohort is the first epidemiologic study to evaluate the association between biomarkers of acrylamide exposure and endometrial cancer risk. We did not observe any evidence to support the hypothesis that levels of biomarkers of acrylamide and glycidamide exposure measured as hemoglobin adducts (HbAA, HbGA, sum of total adducts and HbGA/HbAA ratio) were associated with the risk of developing overall EC or type-I EC in nonsmoking postmenopausal women. Furthermore, there was no evidence for effect measure modification by BMI, alcohol intake, HRT use or OC use though there was relatively limited power to assess heterogeneity among subgroups.

The present study was based on a subgroup of nonsmoking postmenopausal women in the EPIC cohort to address two major concerns. First, tobacco smoking is considered one of the major sources of acrylamide exposure, and it is recognized that smokers have higher levels of acrylamide biomarkers¹⁰; second, hormonal homeostasis may be disrupted by acrylamide,^{7,8} thus, the analyses were performed in nonsmoking postmenopausal women only.

The lack of association between biomarkers of acrylamide exposure and overall and type-I EC risk is in agreement with results we previously reported in the EPIC sub-cohort of women, where hazard ratios were estimated for the association between dietary acrylamide intake (assessed through DQs) and overall EC ($n = 1,382$) or type-I EC risk ($n = 627$); nevertheless, in the full cohort analysis, positive associations were reported between acrylamide intake and type-I EC risk in women who were never smokers and non-users of OCs.¹⁹ In the present study, using circulating biomarkers of acrylamide exposure, we did not replicate these results possibly due to the small sample size with tumor histology information ($n = 171$ type-I EC cases). Additionally, the null results based on FFQ data reported by the Swedish Mammography Cohort study¹⁷ are also in line with the results presented in the current study. However, the Netherlands Cohort Study reported hazard ratios for dietary acrylamide intake and risk of EC of 1.29 (95%CI: 0.81–2.07; p -trend: 0.18) and 1.99 (95%CI: 1.25–3.52; p -trend: 0.03) in the entire cohort and in never

smoking women, respectively.¹⁶ The Nurses' Health Study also reported relative risks for dietary acrylamide intake of 1.41 (95%CI: 1.01–1.97; *p*-trend: 0.03) and 1.43 (95%CI: 0.90–2.28; *p*-trend: 0.04) in the entire cohort and in never smoking women.¹⁸ Two recent meta-analyses concluded that higher consumption of dietary acrylamide was significantly associated with overall EC risk in never smoking women; but not in all women combined.^{20,21} In the present study of acrylamide and glycidamide biomarkers and EC risk in non-smoking postmenopausal women, we did not observe any evidence for associations with overall or type-I EC risk.

The main strengths of the present nested case-control study are its study design, with the intention to prevent confounding from tobacco smoking and hormonal fluctuations, and the use of prospective information on the main risk factors for EC. The minimum detectable ORs at 80% power in our study were 1.22 and 1.60 for the continuous and categorical variables, respectively. Moreover, measurement errors from using acrylamide intake estimates based on FFQs were avoided, and the quantification of HbAA and HbGA was performed following rigorous quality assurance/quality control laboratory protocols¹⁰; and all blood samples were drawn from participants before disease diagnosis. The present study also had limitations: (a) a single blood sample was collected at baseline for each observation, thus, we were not able to measure intra-individual variability in adduct measurements. Hemoglobin adducts of acrylamide and glycidamide reflect exposure to acrylamide within the past 4 months, thus, a single measurement may not capture long-term average exposure in the presence of high intra-individual variability. In a

small study of 13 participants Vikström *et al.* observed high intra-individual variability (up to twofold and fourfold differences in HbAA and HbGA levels, respectively) over a period of 20 months.²⁵ By contrast, the NHS-II study observed lower intra-individual variability for Hb-adduct measurements (intra-individual correlation = 0.78, 0.80, and 0.77 for HbAA, HbGA and sum of HbAA+HbGA, respectively) from 45 nonsmoking women at two time-points separated by a median of 23 months.²⁶ (b) Although all models accounted for matching variables as well as known EC risk factors, we cannot exclude the possibility of residual confounding in our analyses. (c) Further, variables for second-hand smoke (SHS) exposure could not be evaluated in statistical models due to the large number of missing values (>50%). In a subset of the present study with available data, no statistically significant differences in Hb-adducts levels were observed between controls who reported not being exposed to SHS (*n* = 80) and controls who were exposed to SHS (*n* = 53) (data not presented). Moreover, two additional studies reported null or negligible effects of SHS on biomarkers of acrylamide exposure.^{27,28} (d) Despite having information on tumor histology for 97% of the EC cases (of which 46% were classified as type-I), we were not able to analyze type-II EC due to the small sample size (*n* = 14).

In conclusion, this study does not provide evidence of an association between levels of hemoglobin adducts of acrylamide and glycidamide and risks of overall EC and type-I EC.

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References

1. IARC. IARC working group on the evaluation of carcinogenic risks to humans: some industrial chemicals. Lyon, 15–22 February 1994. *IARC Monogr EvalCarcinogRisks Hum* 1994;60: 1–560.
2. Tareke E, Rydberg P, Karlsson P, et al. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem* 2002;50:4998–5006.
3. Friedman M. Chemistry, biochemistry, and safety of acrylamide: a review. *J Agric Food Chem* 2003; 51:4504–26.
4. Becalski A, Brady B, Feng S, et al. Formation of acrylamide at temperatures lower than 100°C: the case of prunes and a model study. *Food Addit Contam A Chem Anal Control Expo Risk Assess* 2011;28:726–30.
5. Freisling H, Moskal A, Ferrari P, et al. Dietary acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition differs greatly according to geographical region. *Eur J Nutr* 2013;52:1369–80.
6. LoPachin RM, Gavin T. Acrylamide-induced nerve terminal damage: relevance to neurotoxic and neurodegenerative mechanisms. *J Agric Food Chem* 2008;56:5994–6003.
7. Hogervorst JG, Fortner RT, Mucci LA, et al. Associations between dietary acrylamide intake and plasma sex hormone levels. *Cancer Epidemiol Biomarkers Prev* 2013;22:2024–36.
8. Nagata C, Konishi K, Tamura T, et al. Associations of acrylamide intake with circulating levels of sex hormones and prolactin in premenopausal Japanese women. *Cancer Epidemiol Biomarkers Prev* 2015;24:249–54.
9. Fennell TR, Sumner SC, Walker VE. A model for the formation and removal of hemoglobin adducts. *Cancer Epidemiol Biomarkers Prev* 1992;1:213–9.
10. Vesper HW, Slimani N, Hallmans G, et al. Cross-sectional study on acrylamide hemoglobin adducts in subpopulations from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *J Agric Food Chem* 2008;56: 6046–53.
11. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015;65:87–108.
12. Amant F, Moerman P, Neven P, et al. Endometrial cancer. *Lancet* 2005;366:491–505.
13. Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: more than two types. *Lancet Oncol* 2014;15:e268–78.
14. Cote ML, Alhaji T, Ruterbusch JJ, et al. Risk factors for endometrial cancer in black and white women: a pooled analysis from the epidemiology of endometrial cancer consortium (E2C2). *Cancer Causes Control* 2015;26:287–96.
15. Merritt MA, Tzoulaki I, Tworoger SS, et al. Investigation of dietary factors and endometrial cancer risk using a nutrient-wide association study approach in the EPIC and nurses' health study (NHS) and NHSII. *Cancer Epidemiol Biomarkers Prev* 2015;24:466–71.
16. Hogervorst JG, Schouten LJ, Konings EJ, et al. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev* 2007; 16:2304–13.
17. Larsson SC, Hakansson N, Akesson A, et al. Long-term dietary acrylamide intake and risk of endometrial cancer in a prospective cohort of Swedish women. *Int J Cancer* 2009;124:1196–9.
18. Wilson KM, Mucci LA, Rosner BA, et al. A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancers. *Cancer Epidemiol Biomarkers Prev* 2010;19: 2503–15.
19. Obón-Santacana M, Kaaks R, Slimani N, et al. Dietary intake of acrylamide and endometrial cancer risk in the European prospective investigation into cancer and nutrition cohort. *Br J Cancer* 2014;111:987–97.
20. Pelucchi C, Bosetti C, Galeone C, La Vecchia C. Dietary acrylamide and cancer risk: an updated meta-analysis. *Int J Cancer* 2015;136:2912–22.
21. Je Y. Dietary acrylamide intake and risk of endometrial cancer in prospective cohort studies. *Arch Gynecol Obstet* 2014;291:1395–401.

22. Riboli E, Hunt KJ, Slimani N, et al. European prospective investigation into cancer and nutrition (EPIC): study populations and data collection. *Public Health Nutr* 2002;5:1113–24.
23. Cust AE, Kaaks R, Friedenreich C, et al. Metabolic syndrome, plasma lipid, lipoprotein and glucose levels, and endometrial cancer risk in the European prospective investigation into cancer and nutrition (EPIC). *Endocr Relat Cancer* 2007;14:755–67.
24. Wareham NJ, Jakes RW, Rennie KL, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Public Health Nutr* 2003;6:407–13.
25. Vikström AC, Warholm M, Paulsson B, et al. Hemoglobin adducts as a measure of variations in exposure to acrylamide in food and comparison to questionnaire data. *Food Chem Toxicol* 2012;50:2531–9.
26. Wilson KM, Vesper HW, Tocco P, et al. Validation of a food frequency questionnaire measurement of dietary acrylamide intake using hemoglobin adducts of acrylamide and glycidamide. *Cancer Causes Control* 2009;20:269–78.
27. Vesper HW, Bernert JT, Ospina M, et al. Assessment of the relation between biomarkers for smoking and biomarkers for acrylamide exposure in humans. *Cancer Epidemiol Biomarkers Prev* 2007;16:2471–8.
28. Heudorf U, Hartmann E, Angerer J. Acrylamide in children—exposure assessment via urinary acrylamide metabolites as biomarkers. *Int J Hyg Environ Health* 2009;212:135–41.

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Acrylamide and Glycidamide Hemoglobin Adducts and Epithelial Ovarian Cancer: A Nested Case–Control Study in Nonsmoking Postmenopausal Women from the EPIC Cohort

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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Abstract

Background—Acrylamide was classified as “probably carcinogenic to humans (group 2A)” by the International Agency for Research on Cancer. Epithelial ovarian cancer (EOC) is the fourth cause of cancer mortality in women. Five epidemiological studies have evaluated the association

between EOC risk and dietary acrylamide intake assessed using food frequency questionnaires, and one nested case–control study evaluated hemoglobin adducts of acrylamide (HbAA) and its metabolite glycidamide (HbGA) and EOC risk; the results of these studies were inconsistent.

Methods—A nested case–control study in nonsmoking post-menopausal women (334 cases, 417 controls) was conducted within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Unconditional logistic regression models were used to estimate ORs and 95% confidence intervals (CI) for the association between HbAA, HbGA, HbAA+HbGA, and HbGA/HbAA and EOC and invasive serous EOC risk.

Results—No overall associations were observed between biomarkers of acrylamide exposure analyzed in quintiles and EOC risk; however, positive associations were observed between some middle quintiles of HbGA and HbAA+HbGA. Elevated but non-statistically significant ORs for serous EOC were observed for HbGA and HbAA+HbGA (OR_{Q5vsQ1}, 1.91; 95% CI, 0.96–3.81 and OR_{Q5vsQ1}, 1.90; 95% CI, 0.94–3.83, respectively); however, no linear dose–response trends were observed.

Conclusion—This EPIC nested case–control study failed to observe a clear association between biomarkers of acrylamide exposure and the risk of EOC or invasive serous EOC.

Impact—It is unlikely that dietary acrylamide exposure increases ovarian cancer risk; however, additional studies with larger sample size should be performed to exclude any possible association with EOC risk.

Introduction

In 1994, the International Agency for Research on Cancer (IARC) classified acrylamide as “probably carcinogenic to humans (group 2A).” Acrylamide is formed in carbohydrate rich foods during common cooking procedures such as frying, baking, or roasting, which involve temperatures usually higher than 120°C (1, 2).

Acrylamide is thought to be absorbed in the gastrointestinal tract mainly through passive transport, and once it is in the body, is metabolized by at least two pathways: via direct conjugation with glutathione for its elimination, or via the Cyp2e1 enzyme system to form glycidamide, a DNA-reactive epoxide (3). Both acrylamide and glycidamide can interact with hemoglobin to form adducts (HbAA and HbGA, respectively) which are considered relevant biomarkers of internal exposure, represent exposure over the life-span of erythrocytes, previous ≈4 months (4, 5), and have been used in multiple epidemiological and experimental studies. In addition to dietary acrylamide intake, tobacco smoking, occupational exposures, and environmental tobacco smoke can also influence levels of HbAA and HbGA (6). It has been observed that smokers have, on average, three to four times higher levels of hemoglobin adducts than nonsmokers (7).

Genotoxic and mutagenic properties have been described in animals after glycidamide exposure. Furthermore, several animal studies observed an increase in the incidence of hormone and nonhormone-related tumors after acrylamide exposure (8).

Almost 90% of malignant ovarian tumors are epithelial ovarian cancer (EOC), which is the seventh most common cancer in women worldwide, and the fourth cause of cancer mortality in women (9). The 5-year survival rate ranges between 30% and 50% depending upon geographic region (10). There is epidemiological evidence that both adult attained height and body mass index (BMI) increase the risk of developing EOC (11, 12), and that tobacco smoking is positively associated with mucinous ovarian cancer (13, 14), whereas oral contraceptive (OC) use and full-term pregnancy are established preventive factors (15).

Four prospective cohort studies and one case–control study have evaluated the association between dietary acrylamide intake (assessed using food frequency questionnaires, FFQ) and EOC risk (16–20). A lack of association was reported in an Italian case–control study (20), the prospective Swedish Mammography Cohort (SMC; ref. 17), and the EPIC cohort (19). The Nurses’ Health Study (NHS) observed a nonstatistically significant increased risk only for serous EOC tumors (18). Nevertheless, a prospective study within the Netherlands Cohort Study (NLCS) observed a statistically significant positive association between high consumption of acrylamide and overall EOC risk (16). A nested case–control study was subsequently conducted within the NHS and the NHSII (NHS/NHSII) to examine the relation between acrylamide exposure measured as hemoglobin adducts and EOC risk (21); however, no evidence for any associations for overall EOC or serous EOC risk were observed comparing the highest to the lowest tertile of HbAA and HbGA.

The present nested case–control study was performed in a subgroup of nonsmoking postmenopausal women from the EPIC cohort with the aim to evaluate the association between EOC risk and hemoglobin adducts of acrylamide/glycidamide. Analyses by different EOC histologic subtype and tumor invasiveness were also performed, as well as stratified analyses by known risk and preventive factors in the development of EOC.

Materials and Methods

Study population and data collection

The EPIC study is an ongoing multicenter prospective cohort study which comprises 23 research centers in 10 European countries (France, Italy, Spain, the United Kingdom, The Netherlands, Greece, Germany, Sweden, Denmark, and Norway). Norway, Denmark, and a center from Sweden (Malmo) did not participate in the present nested case–control study. All EPIC study participants signed an informed consent at recruitment (range: 1992–2000), and the study was approved by both the ethical review boards from the IARC, and local ethics committees. Details of the study methodology have been previously described (22).

The EPIC study includes 153,427 men and 367,903 women. At recruitment, participants completed country-specific, validated dietary questionnaires (DQ) with the time frame referring to the previous year. Information on lifestyle factors (such as tobacco smoking, level of education, socioeconomic status, alcohol consumption, and physical activity), anthropometric factors, brief occupational history, and medical history were also assessed at recruitment. Women also reported baseline information on menstrual and reproductive factors [i.e., age at first menstrual period, pregnancy, use of OC, use of hormone replacement therapy (HRT), and menopausal status].

The standardized protocol followed to collect and store blood samples at recruitment has been previously published (22). Briefly, almost 80% of the EPIC participants, of which 226,673 were women, provided a single blood sample. Most of the samples were stored in liquid nitrogen (-196°C) at the IARC bio-bank; however, samples from Sweden (Umeå) were stored in freezers (-80°C) at the Medical Biobank of Northern Sweden.

Identification of epithelial ovarian cancer cases and selection of the study population

Incident EOC were classified according to the International Classification of Diseases for Oncology (ICD-0-3), and included epithelial borderline tumors (C56.9), invasive epithelial ovarian (C56.9), fallopian tube (C57.0), and primary peritoneal (C48) cancers. Incident EOC were recorded through a combination of methods (health insurance records, cancer and pathology registries, and active follow-up), or via population cancer registries.

Cases and controls for the present nested case-control study were selected according to the methodology described by Peeters and colleagues (23). To summarize, for each case (participant who developed an ovarian, fallopian tube, or peritoneal tumor after the date of blood draw and before the end of follow-up) two controls free of cancer (with the exception of nonmelanoma skin cancer) were randomly selected at the time of diagnosis using a density sampling protocol. Matching criteria included study center, menopausal status (premenopausal, postmenopausal, perimenopausal), age at recruitment (± 6 months), time of the day of blood collection (± 1 hour), and fasting status (< 3 , $3-6$, > 6 hours). For the current study of hemoglobin adducts, one control per case was selected. Because acrylamide may disrupt hormonal levels, and tobacco smoking is an important source of acrylamide exposure (7, 24, 25), this study only included women who at baseline reported being postmenopausal and nonsmokers (thus, individual matching was broken). Postmenopausal women were defined as those who were > 55 years old, or who reported not having had any menses during the 12 months before recruitment. Nonsmokers women were defined as those who reported never smoking or having given up smoking ≥ 5 years before recruitment.

A total of 751 participants (334 EOC cases and 417 controls) were included in the study. EOC comprised both borderline ($n = 2$, 1%) and invasive tumors ($n = 332$, 99%). Invasive EOC were classified into subtypes: serous ($n = 191$, 58%), endometrioid ($n = 26$, 8%), mucinous ($n = 18$, 5%), clear cell ($n = 12$, 3%), not otherwise specified (NOS) which included adenocarcinomas, carcinomas, and cystadenocarcinoma ($n = 79$, 24%), and others ($n = 6$, 2%).

Measurement of acrylamide and glycidamide hemoglobin adducts

Blood samples were sent to the Center for Disease Control and Prevention (CDC) Protein Biomarker Laboratory (Atlanta, USA) to measure HbAA and HbGA. Details of the methodology can be found elsewhere (7, 26). Briefly, 300 mL of red blood cells were hemolyzed and analyzed using HPLC/tandem mass spectrometry (HPLC/MS-MS). Laboratory personnel were blinded to the case-control status of participants, and blood samples were analyzed in a randomized manner. Concentrations of HbAA and HbGA were reported relative to the amount of hemoglobin (pmol per g of Hb), and two independent measures were performed for each sample. The lower limits of detection for this method are

3 pmol/g of Hb for HbAA, and 4 pmol/g of Hb for HbGA. All of the HbAA and HbGA measurements were within the limits of detection. In this study, 42 of the 751 blood samples were sent in duplicate to the laboratory to independently assess the reproducibility of the hemoglobin adduct measures, which had intraclass correlation coefficients of 0.94 for HbAA and 0.92 for HbGA. The percent coefficient of variation (CV) was estimated using log-transformed (\log_2) values, and was 9.9 for HbAA and 12.0 for HbGA.

Statistical methods

Unconditional logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association between biomarkers levels of acrylamide and glycidamide and the risk of EOC. Conditional logistic regression model were also evaluated in a sensitivity analyses.

All statistical models were adjusted for matching factors [age at recruitment (in years), country, time of the day of blood draw, date of blood draw, and fasting status] and covariates including OC use (never, ever, unknown), HRT use (never, ever, unknown), alcohol consumption (nondrinkers, drinkers of 0–6, >6–12, >12–24, and >24 g/day), parity (nulliparous, 1, 2, ≥ 3 , parous but with missing number of full-term pregnancies), age at menopause (years), age at first menstrual period (years), and BMI (kg/m^2). Lifestyle, anthropometric, and reproductive variables such as physical activity using the Cambridge index (27), education level (none, primary, technical/professional, secondary, and higher education), height (cm), weight (kg), hip circumference (cm), waist circumference (cm), duration of using OC (years), duration of using HRT (years), and age at first birth (years) were evaluated as potential confounders, but were not included in final models because they did not change effect estimates $>10\%$.

Restricted cubic splines with 3, 4, and 5 knots were evaluated, and indicated nonmonotonic relations between each of the four biomarker variables and EOC risk. Because the relations were not linear, even when exposure variables were logarithmically (\log_2) transformed, results for continuous biomarker variables were not presented (28). For each biomarker quintile, the median was estimated, and was included in a score test to evaluate dose–response trends. The four continuous biomarker variables HbAA, HbGA, sum of total adducts (HbAA+HbGA) and HbGA/HbAA ratio were categorized into quintiles based on the exposure distribution in controls. Biomarker quartiles were evaluated in stratified analyses.

Analyses were also carried out excluding borderline tumors ($n = 2$), and by histologic subtypes: invasive serous EOC, invasive serous EOC combined with NOS, and nonserous EOC (which included endometrioid, mucinous, clear cell, and NOS tumors).

Effect measure modification was evaluated by BMI ($<25 \text{ kg/m}^2$ vs. $\geq 25 \text{ kg/m}^2$), HRT (never vs. ever users), OC (never vs. ever users), and alcohol intake (never vs. ever drinkers) using a likelihood ratio test (LRT). These variables were selected because they are established risk or preventive factors, or because they may affect the activity of Cyp2e1 (29). All statistical tests were two-sided and evaluated at α -level 0.05. All analyses were performed using SAS v. 9.1.

Results

Description of the study population

The present nested case–control study was based on 334 incident EOC cases (of which 191 were classified as serous) and 417 controls. A large proportion of cases and controls were from the United Kingdom and the Netherlands (Table 1). Among cases, the median (quartile range) of HbAA and HbGA levels were 42.2 (33.9–54.4) and 37.0 (28.5–49.5), respectively, whereas controls had HbAA and HbGA levels of 43.1 (33.8–54.8) and 35.4 (26.0–49.9), respectively (Table 1). Cases were slightly younger than controls (58.4 years vs. 59.2 years), tended to have higher BMI values (26.4 vs. 25.8 kg/m²), a higher proportion of HRT users (27.8% vs. 18.9%), and were less likely to take OC (35.6% vs. 41.7%). There were no major differences between cases and controls regarding age at menopause, age at first menstrual period, and parity (Table 1). The median interval between the date of blood draw and the date at diagnosis for cases was 6.2 years.

Overall EOC and serous EOC risk

Four multivariate unconditional logistic regression analyses were performed for the association between each biomarker exposure variable and EOC risk. No associations were observed between HbAA levels analyzed in quintiles and EOC risk. Participants with HbGA levels >52.71 pmol/g of Hb (fifth quintile) were at nonsignificant increased EOC risk (OR_{Q5vsQ1}, 1.63; 95% CI, 0.92–2.86). The sum of total adducts was also analyzed. Compared to women with ≤56.70 pmol/g of Hb (reference group), the ORs for the fourth and fifth quintiles were elevated but none were statistically significant. Participants classified in the second and third quintile of HbAA+HbGA were at higher risk of developing EOC (OR_{Q2vsQ1}, 1.81; 95% CI, 1.06–3.10) and (OR_{Q3vsQ1}, 2.00; 95% CI, 1.16–3.45).

Similar models were also evaluated for invasive serous EOC. Despite not observing any statistically significant associations between biomarker levels (HbAA, HbGA, HbAA +HbGA, and HbGA/HbAA) and serous EOC risk, positive nonstatistically significant associations were observed for upper versus lower quintiles of HbGA and HbAA+HbGA (Table 2). Similar patterns were found when borderline tumors were excluded, when nonserous tumors were evaluated, and when invasive serous and NOS were combined in the same analyses (data not shown).

Sensitivity analyses were conducted using conditional logistic regression models, which included 261 cases and 416 controls, to estimate ORs of EOC for each biomarker level. Overall, no statistically significant association were observed; nonetheless, results showed similar patterns compared to the ones obtained using unconditional logistic regression models (Table 2).

Effect measure modification in EOC

Although some individual ORs were statistically significant, no consistent evidence for effect measure modification by BMI, alcohol intake, OC use (all LRT *P*-values >0.07; Table 3), or by HRT use (data not shown) was observed.

Discussion

The present nested case–control study was performed to assess the association between circulating hemoglobin adducts of acrylamide and glycidamide exposure and the risk of EOC in non-smoking postmenopausal women from the EPIC cohort. Overall, our results do not support the hypothesis of an association between acrylamide or glycidamide biomarker levels and EOC risk; although increased risks were observed for some middle quintiles of HbGA and HbAA+HbGA, and nonstatistically significant increased risk for serous EOC was observed for the fifth versus the first quintile of HbGA and HbAA+HbGA. No evidence for effect measure modification was noted when subgroups were analyzed.

Acrylamide is thought to be carcinogenic through its reactive epoxide, glycidamide, which forms DNA adducts and induces tumor development in animal models (30). Epidemiologic evidence for an association between dietary acrylamide consumption and EOC risk is controversial. Only two of the five published studies (four prospective studies and one case–control study) found positive associations or suggestive increased risks for the relation between acrylamide (measured using FFQs) and overall EOC or serous EOC (16, 18). The main results of the present nested case–control study are in line with the results presented in the Italian case–control, the SMC, and the EPIC cohort study (17, 19, 20).

A previous nested case–control biomarker study (conducted within the NHS and the NHSII) also concluded that there were no associations between adduct levels (measured as HbAA, HbGA, and HbAA+HbGA) and EOC or serous EOC risk (21). However, most of the effect estimates presented in the NHS/NHSII study were below the null value; unlike those observed in the current EPIC study. Moreover, the NHS/NHSII study included participants who were pre- or perimenopausal, and current or former smokers, whereas this study was based on postmenopausal non-smoking women, because our aim was to evaluate the effect of dietary acrylamide exposure, and tobacco smoking is widely recognized to influence hemoglobin adduct concentrations (7, 31).

Blood samples from both EPIC and the NHS/NHSII studies were measured in the same laboratory using the same protocol. Among cases, the median adducts levels presented in the NHS/NHSII study were 63.8, 49.5, and 112.6 pmol/g Hb, whereas in this study median adducts levels were lower at 42.2, 37.0, and 79.3 pmol/g Hb for HbAA, HbGA, and HbAA +HbGA, respectively. To avoid possible confounding by tobacco smoking, the NHS/NHSII study restricted the analyses to nonsmoking women at the time of blood extraction (230 cases vs. 460 controls), and categorized exposures in tertiles based on the distribution in nonsmoking controls; however, referent group cutpoints were higher for HbAA, HbGA, and HbAA+HbGA (0–52.3, 0–40.2, and 0–95.7 pmol/g Hb, respectively) compared with those presented in this study (≤ 36.5 , ≤ 29.6 , and ≤ 66.2 pmol/g Hb, respectively; tertile data not shown in tables). The minimum detectable OR_{Q5} at 80% power in our study was 1.65, which is similar to the minimum detectable OR (1.78) reported by the NHS/NHSII study.

The design of the present nested case–control study is one of the major strengths, as we wanted to evaluate the dietary contribution to acrylamide biomarker levels and EOC risk, and avoid confounding from tobacco smoking and hormonal oscillations. Dietary

acrylamide exposure assessment using FFQs has been criticized due to its low correlation with hemoglobin adducts of exposure in many epidemiologic studies (correlation range: 0.08–0.43; ref. 19); however, this weakness was avoided because our exposure data were based on hemoglobin adducts levels. Furthermore, HbAA and HbGA levels were measured in blood collected before cancer diagnosis, and following exhaustive quality assurance and quality control laboratory protocols (7, 26). There are some limitations that should be noted: (i) only one blood sample was collected at baseline from each participant, and this did not allow us to estimate intra-individual variation; however, a prior study conducted in 45 women from the NHS-II (who provided two to three blood samples over a period of 1–3 years) suggested that biomarkers of acrylamide intake were reproducible over time (32), (ii) although the EPIC study has prospective information for most of the known EOC risks factors, information on endometriosis and polycystic ovary syndrome could not be accounted for in our statistical analyses since it was not collected, (iii) occupational exposure and environmental tobacco smoke exposure could not be evaluated due to the large number of missing values (>50%) for environmental tobacco smoke, and the low prevalence of occupational exposure information in women, (iv) and despite having a larger number of EOC cases ($n = 334$) than the NHS/NHSII study ($n = 263$), we were unable to perform analyses for EOC subtypes other than serous due to small sample size.

In summary, this nested case–control study within the EPIC cohort failed to observe a clear association between biomarkers of acrylamide exposure (measured as hemoglobin adducts of acrylamide and glycidamide in red blood cells) and the risk of EOC or serous EOC. Additional studies with larger sample size, and pooled analysis of existing studies should be performed to exclude any possible association.

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References

1. Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M. Acrylamide: a cooking carcinogen? *Chem Res Toxicol*. 2000; 13:517–22. [PubMed: 10858325]

2. Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem.* 2002; 50:4998–5006. [PubMed: 12166997]
3. Zötl B, Schmid D, Wassler G, Gundacker C, Leibetseder V, Thalhammer T, et al. Intestinal transport and metabolism of acrylamide. *Toxicology.* 2007; 232:99–108. [PubMed: 17267090]
4. Friedman M. Chemistry, biochemistry, and safety of acrylamide: a review. *J Agric Food Chem.* 2003; 51:4504–26. [PubMed: 14705871]
5. Fennell TR, Sumner SC, Walker VE. A model for the formation and removal of hemoglobin adducts. *Cancer Epidemiol Biomarkers Prev.* 1992; 1:213–9. [PubMed: 1306107]
6. Vesper HW, Caudill SP, Osterloh JD, Meyers T, Scott D, Myers GL. Exposure of the U.S. population to acrylamide in the National Health and Nutrition Examination Survey 2003–2004. *Environ Health Perspect.* 2010; 118:278–83. [PubMed: 20123601]
7. Vesper HW, Slimani N, Hallmans G, Tjønneland A, Agudo A, Benetou V, et al. Cross-sectional study on acrylamide hemoglobin adducts in sub-populations from the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. *J Agric Food Chem.* 2008; 56:6046–53. [PubMed: 18624432]
8. Hogervorst JG, Baars BJ, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. *Crit Rev Toxicol.* 2010; 40:485–512. [PubMed: 20170357]
9. Ferlay, J., Soerjomataram, I., Ervik, M., Dikshit, R., Eser, S., Mathers, C., et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. [cited 2015 Jun 30]. Available from: <http://globocan.iarc.fr>
10. World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Report. Food, Nutrition, Physical Activity, and the Prevention of Ovarian Cancer. 2014. [Internet]. [cited 2015 May 5]. Available from: http://www.dietandcancerreport.org/cup/cup_resources.php
11. Aune D, Navarro Rosenblatt DA, Chan DSM, Abar L, Vingeliene S, Vieira AR, et al. Anthropometric factors and ovarian cancer risk: a systematic review and nonlinear dose-response meta-analysis of prospective studies. *Int J Cancer.* 2015; 136:1888–98. [PubMed: 25250505]
12. Lahmann PH, Cust AE, Friedenreich CM, Schulz M, Lukanova A, Kaaks R, et al. Anthropometric measures and epithelial ovarian cancer risk in the European Prospective Investigation into Cancer and Nutrition. *Int J Cancer.* 2010; 126:2404–15. [PubMed: 19821492]
13. Gram IT, Lukanova A, Brill I, Braaten T, Lund E, Lundin E, et al. Cigarette smoking and risk of histological subtypes of epithelial ovarian cancer in the EPIC cohort study. *Int J Cancer.* 2012; 130:2204–10. [PubMed: 21678398]
14. Secretan B, Straif K, Baan R, Grosse Y, El Ghissassi F, Bouvard V, et al. A review of human carcinogens. Part E: Tobacco, areca nut, alcohol, coal smoke, and salted fish. *Lancet Oncol.* 2009; 10:1033–4. [PubMed: 19891056]
15. Fortner RT, Ose J, Merritt MA, Schock H, Tjønneland A, Hansen L, et al. Reproductive and hormone-related risk factors for epithelial ovarian cancer by histologic pathways, invasiveness and histologic subtypes: Results from the EPIC cohort. *Int J Cancer.* 2015; 137:1196–208. [PubMed: 25656413]
16. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev.* 2007; 16:2304–13. [PubMed: 18006919]
17. Larsson SC, Akesson A, Wolk A. Long-term dietary acrylamide intake and risk of epithelial ovarian cancer in a prospective cohort of Swedish women. *Cancer Epidemiol Biomarkers Prev.* 2009; 18:994–7. [PubMed: 19223560]
18. Wilson KM, Mucci LA, Rosner BA, Willett WC. A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancers. *Cancer Epidemiol Biomarkers Prev.* 2010; 19:2503–15. [PubMed: 20693310]
19. Obón-Santacana M, Peeters PHM, Freisling H, Dossus L, Clavel-Chapelon F, Baglietto L, et al. Dietary intake of acrylamide and epithelial ovarian cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. *Cancer Epidemiol Biomarkers Prev.* 2015; 24:291–7. [PubMed: 25300475]

20. Pelucchi C, Galeone C, Levi F, Negri E, Franceschi S, Talamini R, et al. Dietary acrylamide and human cancer. *Int J Cancer*. 2006; 118:467–71. [PubMed: 16003724]
21. Xie J, Terry KL, Poole EM, Wilson KM, Rosner BA, Willett WC, et al. Acrylamide hemoglobin adduct levels and ovarian cancer risk: a nested case-control study. *Cancer Epidemiol Biomarkers Prev*. 2013; 22:653–60. [PubMed: 23417989]
22. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr*. 2002; 5:1113–24. [PubMed: 12639222]
23. Peeters PH, Lukanova A, Allen N, Berrino F, Key T, Dossus L, et al. Serum IGF-I, its major binding protein (IGFBP-3) and epithelial ovarian cancer risk: the European Prospective Investigation into Cancer and Nutrition (EPIC). *Endocr Relat Cancer*. 2007; 14:81–90. [PubMed: 17395977]
24. Hogervorst JG, Fortner RT, Mucci LA, Tworoger SS, Eliassen AH, Hankinson SE, et al. Associations between dietary acrylamide intake and plasma sex hormone levels. *Cancer Epidemiol Biomarkers Prev*. 2013; 22:2024–36. [PubMed: 23983241]
25. Nagata C, Konishi K, Tamura T, Wada K, Tsuji M, Hayashi M, et al. Associations of acrylamide intake with circulating levels of sex hormones and prolactin in premenopausal Japanese women. *Cancer Epidemiol Biomarkers Prev*. 2015; 24:249–54. [PubMed: 25352525]
26. Vesper HW, Ospina M, Meyers T, Ingham L, Smith A, Gray JG, et al. Automated method for measuring globin adducts of acrylamide and glycidamide at optimized Edman reaction conditions. *Rapid Commun Mass Spectrom*. 2006; 20:959–64. [PubMed: 16479554]
27. Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Public Health Nutr*. 2003; 6:407–13. [PubMed: 12795830]
28. Heinzl H, Kaider A. Gaining more flexibility in Cox proportional hazards regression models with cubic spline functions. *Comput Methods Programs Biomed*. 1997; 54:201–8. [PubMed: 9421665]
29. Freisling H, Moskal A, Ferrari P, Nicolas G, Knaze V, Clavel-Chapelon F, et al. Dietary acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition differs greatly according to geographical region. *Eur J Nutr*. 2013; 52:1369–80. [PubMed: 23238529]
30. Mei N, McDaniel LP, Dobrovolsky VN, Guo X, Shaddock JG, Mittelstaedt RA, et al. The genotoxicity of acrylamide and glycidamide in big blue rats. *Toxicol Sci*. 2010; 115:412–21. [PubMed: 20200216]
31. Spivey A. A matter of degrees: advancing our understanding of acrylamide. *Environ Health Perspect*. 2010; 118:A160–7. [PubMed: 20359976]
32. Wilson KM, Vesper HW, Tocco P, Sampson L, Rosen J, Hellenas KE, et al. Validation of a food frequency questionnaire measurement of dietary acrylamide intake using hemoglobin adducts of acrylamide and glycidamide. *Cancer Causes Control*. 2009; 20:269–78. [PubMed: 18855107]

Table 1

Description of the study population from a nested case–control study of acrylamide biomarkers and EOC in the EPIC cohort

	All EOC cases <i>n</i> = 334	Invasive serous EOC cases <i>n</i> = 191	Controls <i>n</i> = 417
HbAA pmol/g of Hb ^a	42.2 (33.9–54.4)	42.2 (33.8–56.6)	43.1 (33.8–54.8)
HbGA pmol/g of Hb ^a	37.0 (28.5–49.5)	37.0 (28.1–52.2)	35.4 (26.0–49.9)
HbAA+HbGA pmol/g of Hb ^a	79.3 (62.5–105.4)	82.1 (62.0–107.8)	78.7 (60.6–106.0)
HbGA/HbAA pmol/g of Hb ^a	0.9 (0.7–1.0)	0.9 (0.7–1.0)	0.8 (0.7–1.0)
Age at recruitment (years) ^a	58.4 (53.8–63.4)	57.7 (53.0–62.7)	59.2 (54.4–64.2)
Age at first menstrual period (years) ^a	13.0 (12.0–14.0)	13.0 (12.0–14.0)	13.0 (12.0–14.0)
Age at menopause (years) ^a	49.5 (49.0–52.0)	49.5 (49.0–51.0)	49.5 (48.0–52.0)
BMI (kg/m ²) ^a	26.4 (23.4–29.3)	26.0 (22.8–29.3)	25.8 (23.2–29.5)
Country ^b			
France	32(9.6)	23 (12.0)	30 (7.2)
Italy	43 (12.9)	25 (13.1)	52 (12.5)
Spain	36 (10.8)	21 (11.0)	55 (13.2)
United Kingdom	71 (21.3)	29 (15.2)	94 (22.5)
The Netherlands	59 (17.7)	37 (19.4)	78 (18.7)
Greece	27 (8.1)	10 (5.2)	43 (10.3)
Germany	45(13.5)	33 (17.3)	46 (11.0)
Sweden	21 (6.3)	13 (6.8)	19 (4.6)
Fasting status ^b			
Unknown	3 (0.9)	1 (0.5)	2 (0.5)
<3 hours	169 (50.6)	97 (50.8)	213 (51.1)
3–6 hours	44 (13.2)	23 (12.0)	58 (13.9)
>6 hours	118 (35.3)	70 (36.7)	44 (34.5)
Alcohol consumption ^b			
Non drinker	80 (24.0)	47 (24.6)	93 (22.3)
>0–6	166 (49.7)	95 (49.7)	178 (42.7)
>6–12	35 (10.5)	22 (11.5)	73 (17.5)
>12–24	38 (11.4)	19 (10.0)	50 (12.0)
>24–60	15 (4.5)	8 (4.2)	23 (5.5)
Ever use of OC ^b			
Unknown	6 (1.8)	3 (1.6)	4 (1.0)
No	209 (62.6)	114 (59.7)	239 (57.3)
Yes	119 (35.6)	74 (38.7)	174 (41.7)
Ever use of HRT ^b			
Unknown	12 (3.6)	8 (4.2)	13 (3.1)
No	229 (68.6)	123 (64.4)	325 (77.9)

	All EOC cases <i>n</i> = 334	Invasive serous EOC cases <i>n</i> = 191	Controls <i>n</i> = 417
Yes	93 (27.8)	60 (31.4)	79 (18.9)
Parity ^b			
Unknown	41 (12.3)	27 (14.1)	58 (13.9)
1 child	129 (38.6)	81 (42.4)	161 (38.6)
2 children	99 (29.6)	53 (27.8)	141 (33.8)
≥3 children	48 (14.4)	23 (12.0)	44 (10.6)
Nulliparous	8 (2.4)	4 (2.1)	9 (2.2)
Parous but with missing number of full-term pregnancies	9 (2.7)	3 (1.6)	4 (1.0)

Abbreviation: HbGA, hemoglobin adducts of glycidamide.

^aMedian and quartile range (25th–75th percentile).

^bNumber (*n*) and percent (%).

Table 2
 OR and 95% CI for biomarkers of acrylamide exposure and EOC risk in a nested case-control study in the EPIC cohort

Sensitivity analysis ^b												
Overall EOC ^a						Overall EOC						
Overall EOC ^a			Cases	Controls		Cases	Controls		Cases	Controls		
Exposure cut-points	n = 334	n = 417	P _{trend}	OR (95% CI)		n = 261	n = 416	OR (95% CI)	P _{trend}	n = 191	n = 417	P _{trend}
HbAA												
≤31.30	60	82		1.00 (ref)		45	81	1.00 (ref)		32	82	1.00 (ref)
31.31–39.10	80	85		1.25 (0.75–2.10)		60	85	1.25 (0.71–2.20)		47	85	1.29 (0.68–2.45)
39.11–47.20	60	81		1.01 (0.58–1.76)	0.86	48	81	1.11 (0.61–2.01)	0.94	34	81	0.96 (0.48–1.92)
47.21–59.20	71	86		1.20 (0.69–2.06)		58	86	1.12 (0.63–2.00)		36	86	1.27 (0.65–2.48)
>59.21	63	83		1.19 (0.67–2.11)		50	83	1.04 (0.56–1.93)		42	83	1.55 (0.78–3.09)
HbGA												
≤24.70	51	83		1.00 (ref)		39	82	1.00 (ref)		29	83	1.00 (ref)
24.71–31.30	62	83		1.23 (0.72–2.11)		46	83	1.05 (0.60–1.85)		36	83	1.37 (0.70–2.67)
31.31–41.20	91	84		2.14 (1.27–3.60)	0.04	75	84	1.76 (1.01–3.08)	0.06	49	84	2.11 (1.10–4.03)
41.22–52.70	58	84		1.32 (0.75–2.33)		43	84	0.81 (0.43–1.50)		32	84	1.57 (0.78–3.18)
>52.71	72	83		1.63 (0.92–2.86)		58	83	1.22 (0.66–2.26)		45	83	1.91 (0.96–3.81)
Sum of HbAA + HbGA												
≤56.70	48	83		1.00 (ref)		38	82	1.00 (ref)		28	83	1.00 (ref)
56.71–71.00	77	83		1.81 (1.06–3.10)		54	83	1.41 (0.79–2.52)		43	83	1.67 (0.86–3.26)
71.01–88.90	80	84		2.00 (1.16–3.45)	0.14	68	84	1.77 (0.98–3.19)	0.28	45	84	2.07 (1.06–4.06)
88.91–112.60	64	84		1.75 (0.98–3.13)		49	84	1.09 (0.58–2.02)		33	84	1.68 (0.82–3.44)
>112.61	65	83		1.60 (0.89–2.87)		52	83	1.22 (0.65–2.29)		42	83	1.90 (0.94–3.83)
Ratio of HbGA/HbAA												
≤0.70	55	83		1.00 (ref)		41	83	1.00 (ref)		33	83	1.00 (ref)
0.71–0.79	55	81		1.07 (0.62–1.83)		42	81	0.96 (0.53–1.74)		33	81	1.18 (0.61–2.30)
0.80–0.90	78	89		1.43 (0.85–2.41)	0.46	61	89	1.22 (0.71–2.10)	0.66	43	89	1.43 (0.75–2.74)
0.91–0.99	69	79		1.53 (0.87–2.67)		59	78	1.37 (0.77–2.45)		40	79	1.59 (0.80–3.16)
>1.00	77	85		1.40 (0.82–2.39)		58	85	1.01 (0.56–1.81)		42	85	1.42 (0.74–2.74)

Abbreviation: HbGA, hemoglobin adducts of glycidamide.

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^dModels are adjusted for age at recruitment, country, fasting status, date at blood collection, time of the day of blood collection, OC use, HRT use, alcohol intake, parity, age at menopause, age at first menstrual period, and BMI.

^eConditional logistic regression model adjusting for matching factors and OC use, HRT use, alcohol intake, parity, age at menopause, age at first menstrual period, and BMI.

Table 3

Stratified analyses: OR and 95% CI for biomarkers of acrylamide exposure and EOC risk in an EPIC nested case-control study in the EPIC cohort

Cutpoints	Normal and underweight			Overweight and obese			Never drinkers			Drinkers			Nonoral contraceptive users			Oral contraceptive users		
	Cases	Controls	OR (95% CI) ^a	Cases	Controls	OR (95% CI) ^a	Cases	Controls	OR (95% CI) ^a	Cases	Controls	OR (95% CI) ^a	Cases	Controls	OR (95% CI) ^a	Cases	Controls	OR (95% CI) ^a
HbAA																		
≤33.60	35	37	1.00 (ref)	45	67	1.00 (ref)	19	26	1.00 (ref)	61	78	1.00 (ref)	55	74	1.00 (ref)	24	30	1.00 (ref)
33.61–42.70	35	42	1.01 (0.44–2.33)	55	61	1.15 (0.63–2.09)	27	21	1.35 (0.51–3.52)	63	82	1.11 (0.65–1.92)	55	45	1.47 (0.81–2.68)	32	55	0.80 (0.34–1.89)
42.71–54.60	28	50	0.68 (0.29–1.59)	56	55	1.52 (0.81–2.87)	19	28	0.65 (0.22–1.96)	65	77	1.40 (0.80–2.43)	55	58	1.22 (0.66–2.26)	27	46	1.12 (0.44–2.88)
>54.61	39	46	1.23 (0.54–2.84)	41	59	0.93 (0.47–1.82)	15	18	0.77 (0.24–2.52)	65	87	1.19 (0.67–2.12)	44	62	0.85 (0.44–1.66)	36	43	1.60 (0.64–4.00)
LRT ^d			0.07						0.42						0.18			
HbGA																		
≤25.90	35	49	1.00 (ref)	27	54	1.00 (ref)	11	22	1.00 (ref)	51	81	1.00 (ref)	44	62	1.00 (ref)	16	41	1.00 (ref)
26.91–35.20	38	46	1.66 (0.76–3.60)	51	59	1.45 (0.76–2.78)	16	19	1.85 (0.53–6.53)	73	86	1.47 (0.87–2.49)	50	57	1.22 (0.67–2.24)	37	47	2.91 (1.18–7.14)
35.21–49.90	29	42	1.53 (0.67–3.51)	72	63	2.10 (1.08–4.08)	35	26	4.32 (1.32–14.18)	66	79	1.80 (1.04–3.13)	67	57	2.02 (1.09–3.76)	32	47	2.33 (0.90–5.99)
>49.91	35	38	1.82 (0.79–4.22)	47	66	1.29 (0.64–2.63)	18	26	1.39 (0.38–5.03)	64	78	1.62 (0.91–2.89)	48	63	1.11 (0.57–2.15)	34	39	3.33 (1.27–8.77)
LRT ^d			0.60						0.66						0.33			
Sum of HbAA + HbGA																		
≤59.80	34	41	1.00 (ref)	34	62	1.00 (ref)	11	21	1.00 (ref)	57	82	1.00 (ref)	47	71	1.00 (ref)	19	32	1.00 (ref)
59.81–78.70	34	48	1.12 (0.50–2.49)	62	58	1.85 (1.00–3.43)	28	22	2.80 (0.91–8.59)	68	84	1.30 (0.77–2.22)	57	51	1.60 (0.88–2.92)	37	54	1.59 (0.67–3.80)
78.80–106.00	34	45	1.27 (0.55–2.93)	54	59	1.51 (0.79–2.88)	26	28	1.97 (0.59–6.54)	62	76	1.52 (0.87–2.63)	56	51	1.72 (0.93–3.20)	30	51	1.21 (0.48–3.06)
>106.01	35	41	1.48 (0.63–3.50)	47	63	1.23 (0.63–2.43)	15	22	1.01 (0.27–3.85)	67	82	1.46 (0.83–2.58)	49	66	1.06 (0.55–2.07)	33	37	2.14 (0.83–5.53)
LRT ^d			0.34						0.40						0.29			
Ratio of HbGA/HbAA																		
≤0.70	39	66	1.00 (ref)	28	37	1.00 (ref)	3	15	1.00 (ref)	64	88	1.00 (ref)	39	49	1.00 (ref)	26	54	1.00 (ref)
0.71–0.80	39	48	1.99 (0.96–4.15)	37	55	0.88 (0.43–1.78)	21	21	15.55 (1.74–138.79)	55	82	1.02 (0.61–1.72)	46	59	1.02 (0.54–1.91)	30	44	2.10 (0.90–4.87)
0.81–1.00	39	31	3.06 (1.34–7.01)	65	77	1.22 (0.62–2.39)	29	24	17.90 (2.00–160.05)	75	84	1.48 (0.88–2.50)	69	65	1.79 (0.96–3.32)	31	41	2.21 (0.90–5.45)
>1.01	20	30	1.06 (0.44–2.52)	67	73	1.15 (0.59–2.23)	27	33	11.20 (1.27–99.05)	60	70	1.24 (0.73–2.11)	55	66	1.17 (0.63–2.19)	32	35	1.86 (0.77–4.51)
LRT ^d			0.18						0.09						0.61			

Abbreviation: HbGA, hemoglobin adducts of glycidamide.

^a Adjusted for country, fasting status, date at blood collection, time of the day of blood collection, OC use, HRT use, alcohol intake, parity, age at menopause, and age at first menstrual period.

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^d Adjusted for country, fasting status, date at blood collection, time of the day of blood collection, OC use, HRT use, parity, age at menopause, age at first menstrual period, and BMI.

^e Adjusted for country, fasting status, date at blood collection, time of the day of blood collection, HRT use, alcohol intake, parity, age at menopause, age at first menstrual period, and BMI.

^f All LRT *P*-values for effect measure modification are based on the categorical exposure adduct variable.

Dietary acrylamide and cancer risk: An updated meta-analysis

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The debate on the potential carcinogenic effect of dietary acrylamide is open. In consideration of the recent findings from large prospective investigations, we conducted an updated meta-analysis on acrylamide intake and the risk of cancer at several sites. Up to July 2014, we identified 32 publications. We performed meta-analyses to calculate the summary relative risk (RR) of each cancer site for the highest versus lowest level of intake and for an increment of 10 µg/day of dietary acrylamide, through fixed-effects or random-effects models, depending on the heterogeneity test. Fourteen cancer sites could be examined. No meaningful associations were found for most cancers considered. The summary RRs for high versus low acrylamide intake were 0.87 for oral and pharyngeal, 1.14 for esophageal, 1.03 for stomach, 0.94 for colorectal, 0.93 for pancreatic, 1.10 for laryngeal, 0.88 for lung, 0.96 for breast, 1.06 for endometrial, 1.12 for ovarian, 1.00 for prostate, 0.93 for bladder and 1.13 for lymphoid malignancies. The RR was of borderline significance only for kidney cancer (RR = 1.20; 95% confidence interval, CI, 1.00–1.45). All the corresponding continuous estimates ranged between 0.95 and 1.03, and none of them was significant. Among never-smokers, borderline associations with dietary acrylamide emerged for endometrial (RR = 1.23; 95% CI, 1.00–1.51) and ovarian (RR = 1.39; 95% CI, 0.97–2.00) cancers. This systematic review and meta-analysis of epidemiological studies indicates that dietary acrylamide is not related to the risk of most common cancers. A modest association for kidney cancer, and for endometrial and ovarian cancers in never smokers only, cannot be excluded.

Epidemiological evidence on the relation between dietary acrylamide and the risk of several cancers has continued to accumulate during the last few years, after the publication of our first systematic review and meta-analysis on acrylamide and human cancer.¹ Recently, several data have been released from large cohort studies,^{2–11} including—among others—the European Prospective Investigation into Cancer and Nutrition (EPIC) and the Nurses' Health Study (NHS). In particular, the EPIC study, including over 500,000 participants, reported results for esophageal,² pancreatic³ and endometrial⁴ cancer. An increased risk of esophageal cancer (on the basis of 341 cases) emerged in subjects with intermediate levels as

compared to low acrylamide intake, but no significant association was found for a high intake (hazard ratio, HR = 1.41; 95% confidence interval, CI, 0.86–2.71).² No association emerged for pancreatic cancer, based on 865 incident cases, the HRs being 0.77 (95% CI, 0.58–1.04) for high versus low intake and 0.95 (95% CI, 0.89–1.01) for an increase in acrylamide intake of 10 µg/day.³ The analysis of endometrial cancer, including 1,382 cases—627 of which were type-I—found no overall association with acrylamide (HR = 0.98; 95% CI, 0.78–1.25), but a positive one for type-I endometrial cancer in the subgroup of women who never smoked and never used oral contraceptives (HR = 1.97; 95% CI, 1.08–3.62).⁴

The NHS considered the relation between dietary acrylamide and breast, endometrial and ovarian cancer.⁵ The study included 6,301 cases of breast, 484 of endometrial, and 416 of ovarian cancer. While no association emerged with breast cancer (relative risk, RR = 0.95; 95% CI, 0.87–1.03, for high vs. low intake), the risk was increased for endometrial (RR = 1.41; 95% CI, 1.01–1.97) and, to a lower extent, ovarian cancer (RR = 1.25; 95% CI, 0.88–1.77).⁵ A subsequent nested case-control analysis including data from the NHS I and II considered the relation between acrylamide exposure, as measured by hemoglobin adducts, and ovarian cancer risk, providing no evidence of association (RR = 0.79; 95% CI, 0.50–1.24 for highest vs. lowest tertile of acrylamide adducts).⁶

Our earlier extensive systematic review and meta-analysis on acrylamide and cancer included data of both dietary and occupational exposures published until June 2009.¹ That

Key words: acrylamide, epidemiologic studies, meta-analysis, neoplasms, review

Abbreviations: CI: confidence interval; ER: estrogen receptor; EPIC: European Prospective Investigation into Cancer and Nutrition; HR: hazard ratio; NCS: Netherlands Cohort Study; NHS: Nurses' Health Study; PR: progesterone receptor; RR: relative risk

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What's new?

Acrylamide is formed in a variety of foods, and some evidence suggests it may cause cancer. How dangerous is dietary acrylamide? This study collated data from 32 earlier projects evaluating the relationship between acrylamide consumed in foods and cancer risk. Of fourteen cancer sites represented, only kidney cancer showed a possible increase in risk associated with dietary acrylamide. When they narrowed the analysis to people who had never smoked, dietary acrylamide appeared to slightly increase risk of endometrial and ovarian cancer as well.

meta-analysis found no increase in risk of most types of cancer in relation to acrylamide exposure. Since for several cancer sites the number of cases available from epidemiological studies on dietary acrylamide is now more than doubled, and given the continuing debate on the issue, we updated the quantitative meta-analysis on dietary acrylamide intake and cancer risk.

Material and Methods

The methods used are similar to those described in our earlier meta-analysis.^{1,12} In the current investigation, however, the interest was focused on the estimate of total dietary acrylamide only, because: (i) there are no new relevant data from occupational studies, and (ii) studies considering specific foods/food groups (e.g., fried potatoes and chips, coffee, *etc.*) are no longer relevant, given the accumulating number of investigations providing estimates of total dietary acrylamide. In July 2014, we performed a systematic literature search in the Medline database, using PubMed, without restrictions according to language, using the following search string: (acrylamide OR glycidamide) AND (cancer OR neoplasm OR tumor) AND (diet OR dietary OR food OR foods). Since the literature search of our earlier meta-analysis was conducted in June 2009, we limited our search to the period 2009–2014. A similar search was conducted in EMBASE, using the same keywords and over the same search period. Potentially relevant articles were retrieved and assessed. Those that were not in the scope for this review were excluded (e.g., animal studies, studies aimed at reducing the quantity of acrylamide in specific foods, toxicity studies, studies not focused on cancer risk, *etc.*). Abstract and unpublished studies were also excluded. None of the selected, relevant articles was published in a language other than English. No studies were excluded *a priori* for weakness of design or data quality, and we did not assign quality scores to the studies. A total of 105 publications were identified in the PubMed search and 203 in the EMBASE search. A first selection, mainly based on the title and abstract of the publications, was performed to exclude those studies that were clearly nonrelevant (according to the criteria described above), and 59 publications were retained for further consideration. These were abstracted and reviewed in detail. Among them, 46 did not report original epidemiological results on dietary acrylamide and cancer and were no longer considered, whereas the remaining 13 publications were retained for the review.^{2–5,7–11,13–16} These were

added to the previously selected 19 publications on dietary intake of acrylamide,^{17–35} for a total of 32 publications.

We reviewed all the studies selected and abstracted the following information in a standard format: study design; country; cancer sites; number of cases and person-years (or noncases, or controls), overall and according to each level of acrylamide intake; sex; data categorization (*i.e.*, quartiles or quintiles); mean (or median) acrylamide intake in each category among controls; relative risk estimates (rate ratios, HRs, odds ratios, collectively referred to as RR) and the corresponding 95% CI; and additional results from subgroup analyses.

A few publications reported duplicate results from the same populations. A population-based case-control study conducted in Sweden provided early results on dietary acrylamide intake and risk of cancers of the colorectum, bladder and kidney.³⁰ In a subsequent letter,¹⁷ the authors provided updated results based on a more comprehensive estimate of acrylamide intake (*i.e.*, including additional data on coffee drinking). The latter RRs were thus used for the main meta-analyses. Still, data from the first report³⁰ were used in subgroup analyses on smoking status, as the corresponding results were not available in the subsequent letter. Two publications^{13,20} provided results on acrylamide and colorectal cancer using data from the Netherlands Cohort Study (NCS). We included in the meta-analysis the estimates from the first publication that was based on a larger number of cases and a longer follow-up period.²⁰ Also, two publications from the NCS were focused on breast cancer.^{10,19} We included in the meta-analysis the estimates from the second publication,¹⁰ that had an extended follow-up period and a larger number of cases.

Two publications reported the RRs and 95% CIs as figures only.^{17,18} The exact RR estimates for colorectal, bladder cancer,¹⁷ and for renal cell cancer¹⁸ could be retrieved (Prof. Lorelei Ann Mucci, personal communication) and were used in the meta-analyses, while the RR estimate for kidney cancer¹⁷ and the corresponding 95% CI were no longer available and were thus derived from Figure 2 of Ref. 17 and from Figure 1 of Ref. 18.

When at least two independent risk estimates on dietary acrylamide exposure were available for a specific cancer site, we performed a meta-analysis and calculated pooled RR estimates, and the corresponding 95% CIs, for high versus low level of exposure and for an increment of 10 µg/day of dietary acrylamide. For the latter, when the RR for an increment of 10 µg/day of dietary acrylamide was not available from the

original analysis, we estimated it using a method proposed by Greenland and Longnecker, which relates the natural logarithm of the RR to the corresponding mean value of acrylamide intake across exposure categories,^{36,37} taking into account that risk estimates for subsequent levels of intake are correlated. When data on number of subjects in each level were not available, we ignored the correlation between estimates and calculated the dose-risk slopes using variance-weighted least squares regression.

To obtain the summary RRs for high versus low exposure and, separately, for an increment of 10 µg/day of dietary acrylamide intake, we pooled the corresponding RR estimates according to a fixed-effects model using the inverse variance method (*i.e.*, computing an average effect by weighting the log RR of each study according to the inverse of their sampling variance), when the test for heterogeneity between estimates was not significant (*i.e.*, $p > 0.10$), or to a random-effects model, which considers both within- and between-study variations, using the DerSimonian and Laird method [*i.e.*, using the sum of the inverse of the variance of the log (RR) and the moment estimator of the variance between studies as weights], when the test for heterogeneity between estimates was significant (*i.e.*, $p \leq 0.10$).^{38,39} Heterogeneity between estimates was assessed using the χ^2 test. For cancer sites with at least four published studies, we also computed summary estimates in subgroups of study design (cohort and case-control studies) and smoking habit (when sufficient data were available), and according to menopausal status and hormone receptor status for breast cancer. Given the small number of studies providing relevant results across separate strata, we did not perform meta-analyses on subsets of risk estimates defined according to sex, body mass index or other potential effect modifiers. All the statistical analyses were performed using the STATA package (version 11; StataCorp, College Station, TX).

Results

Table 1 shows the main characteristics and results of the 32 publications that provided data on dietary acrylamide and cancer risk. The publications and their results are ordered by cancer site (according to the International Classification of Diseases) and publication year. Several publications reported risk estimates for more than one cancer site and/or subtype, or separately by sex. Therefore, a total number of 64 estimates for high versus low acrylamide intake and 71 estimates for the continuous measure of intake are given in Table 1. Separate RR estimates for various cancer subtypes (*i.e.*, oral cavity and oro and hypopharynx,¹⁶ or specific lymphoid malignancies⁷) were pooled, in order to obtain a single RR to be included in the meta-analysis of the corresponding cancer site (*i.e.*, oral cavity/pharynx and lymphoid malignancies, respectively).⁴⁰ No meta-analysis was conducted for brain and thyroid cancer (only one estimate available). Therefore, a total number of 55 estimates were included in the meta-

analyses of dietary acrylamide and risk of cancer at various sites.

Table 2 gives, for each cancer site examined, the number of available estimates (at least two) and the corresponding total number of cases, together with the summary RR and 95% CI for both a categorical (*i.e.*, high vs. low level) and a continuous (*i.e.*, for 10 µg/day increase) measure of acrylamide intake. All the RR estimates for high versus low level of intake were close to unity, six were below, seven above unity and one equal to 1.00. Except for kidney cancer (1,802 cases), that showed a borderline significant increased RR (1.20; 95% CI, 1.00–1.45) for high versus low acrylamide intake using a fixed-effects model (p for heterogeneity = 0.16), there was no other significant association. When we repeated the meta-analysis of kidney cancer using a random-effects model, the RR was 1.18 (95% CI, 0.92–1.51). With further reference to kidney cancer, the continuous RR was 1.02 (95% CI, 0.97–1.08), obtained using a random-effects model (p for heterogeneity = 0.03). Using a fixed-effects model, the corresponding RR was 1.02 (95% CI, 0.99–1.06). Breast cancer had the largest number of estimates ($n = 7$) and cases ($n = 16,773$). The corresponding RRs were 0.96 (95% CI, 0.91–1.02) for high versus low intake and 1.00 (95% CI, 0.98–1.01) for an increase in intake of 10 µg/day, with no evidence of heterogeneity between estimates. Six estimates were available for prostate ($n = 13,559$ cases) and colorectal ($n = 6,794$ cases) cancers. As concerns prostate cancer, both RRs were equal to 1.00, while for colorectal cancer the RRs were 0.94 (95% CI, 0.85–1.04) for high versus low intake and 1.00 (95% CI, 0.98–1.01) for an increase of 10 µg/day. For other cancer sites, the number of cases on which the meta-analyses were based was lower. The summary RRs for high versus low acrylamide intake were 0.87 for oral and pharyngeal (933 cases), 1.14 for esophageal (1,546 cases), 1.03 for stomach (787 cases), 0.93 for pancreatic (1,732 cases), 1.10 for laryngeal (707 cases), 0.88 for lung (3,598 cases), 1.06 for endometrial (2,774 cases), 1.12 for ovarian (2,010 cases), 0.93 for bladder (1,838 cases) cancer and 1.13 for lymphoid malignancies (1,208 cases). All the corresponding continuous estimates ranged between 0.95 and 1.03, and none of them was significant. Within most cancer sites, estimates were fairly homogeneous. Significant heterogeneity between estimates was reported only for cancers of the oral cavity and pharynx ($p = 0.08$ for high vs. low intake, $p = 0.07$ for the continuous measure), lung ($p = 0.002$ for high vs. low intake, $p = 0.04$ for the continuous measure), and ovary ($p = 0.06$ for high vs. low intake), besides kidney ($p = 0.03$ for the continuous measure).

Table 3 gives the pooled RRs and 95% CIs of selected cancer sites (*i.e.*, those for which at least four studies were available) for high versus low dietary acrylamide intake, according to subgroups of study design, smoking status (categorized as never vs. ever smokers or never/former vs. current smokers, according to which information was mainly reported in the studies of each cancer site) and, for breast cancer,

Table 1. RR and corresponding 95% CI of specific cancer sites, for high versus low level and for an increase of 10 µg/day of dietary acrylamide intake

Cancer site; first author, reference, year	Study design	No. cases	High vs. low level of intake RR (95% CI)	10 µg/day increase in intake RR (95% CI)	Additional results
Oral cavity and pharynx					
Pelucchi, ³³ 2006	Case-control	749	1.12 (0.76–1.66)	1.01 (0.95–1.07) ¹	
Schouten, ¹⁶ 2009 (Oral cavity) ²	Case-cohort	101	0.72 (0.36–1.42)	0.90 (0.73–1.10)	No heterogeneity by sex. Increased continuous HR in female, but not male, nonsmokers (21 cases)
Schouten, ¹⁶ 2009 (Oro and hypopharynx) ²	Case-cohort	83	0.61 (0.33–1.12)	0.74 (0.53–1.03)	Borderline decreased continuous HR in men. No association in women
Esophagus					
Pelucchi, ³³ 2006	Case-control	395	1.10 (0.65–1.86)	0.99 (0.91–1.09) ¹	
Hogervorst, ²⁰ 2008	Case-cohort	216	0.83 (0.54–1.30)	0.96 (0.85–1.09)	No heterogeneity by smoking, age, physical activity; higher risk for overweight/obese subjects. Comparable results for squamous cell carcinoma and adenocarcinoma
Lin, ¹⁴ 2011 ³	Case-control	594	1.23 (1.02–1.75)	1.04 (0.99–1.09) ¹	Stronger association for esophageal SCC (particularly in nonsmokers) and in overweight/obese subjects
Lujan-Barroso, ² 2014	Cohort	341	1.41 (0.86–2.71)	1.09 (0.96–1.24) ¹	Similar results for esophageal SCC and adenocarcinoma, as well as for never smokers/≥20 years quitters. Lower risk estimates for energy-adjusted acrylamide intake.
Stomach					
Hogervorst, ²⁰ 2008	Case-cohort	563	1.06 (0.78–1.45)	1.02 (0.94–1.10)	No heterogeneity by smoking, age, physical activity, BMI. Comparable results for cardia and noncardia cancer
Hirvonen, ⁹ 2010	Cohort	224	0.96 (0.60–1.53)	1.01 (0.93–1.09) ¹	
Colorectum					
Mucci, ^{17,30} 2003 ^{3,4}	Case-control	591	0.67 (0.4–0.9)	0.92 (0.87–0.98) ¹	No heterogeneity by smoking
Mucci, ²⁹ 2006	Cohort	741	0.9 (0.7–1.3)	1.02 (0.98–1.07) ¹	No heterogeneity by age, BMI
Pelucchi, ³³ 2006	Case-control	2280	0.97 (0.80–1.18)	1.00 (0.97–1.03) ¹	
Hogervorst, ²⁰ 2008	Case-cohort	2190	1.00 (0.84–1.20)	1.00 (0.96–1.06)	No heterogeneity by smoking, age, physical activity, BMI
Larsson, ²⁴ 2009	Cohort	676	0.95 (0.74–1.20)	1.00 (0.95–1.04) ¹	No heterogeneity by smoking
Hirvonen, ⁹ 2010	Cohort	316	0.93 (0.65–1.34)	0.99 (0.93–1.05) ¹	
Hogervorst, ¹³ 2014, men ⁵	Case-cohort	341	1.17 (0.82–1.66)	1.03 (0.94–1.14)	No heterogeneity by smoking. Increased risk in tumors with activating KRAS mutation
Hogervorst, ¹³ 2014, women ⁵	Case-cohort	282	0.76 (0.52–1.11)	0.95 (0.85–1.07)	No heterogeneity by smoking. Decreased risk in tumors with truncating APC mutation
Pancreas					
Hogervorst, ²⁰ 2008	Case-cohort	349	0.98 (0.68–1.40)	1.06 (0.96–1.17)	No heterogeneity by smoking, age, physical activity; higher risk for overweight/obese subjects
Hirvonen, ⁹ 2010	Cohort	192	1.00 (0.62–1.62)	1.01 (0.93–1.09) ¹	
Pelucchi, ¹⁵ 2011	Case-control	326	1.49 (0.83–2.70)	1.01 (0.92–1.10)	No heterogeneity by sex, age, education, BMI, smoking, alcohol drinking and diabetes

Table 1. RR and corresponding 95% CI of specific cancer sites, for high versus low level and for an increase of 10 µg/day of dietary acrylamide intake (Continued)

Cancer site; first author, reference, year	Study design	No. cases	High vs. low level of intake RR (95% CI)	10 µg/day increase in intake RR (95% CI)	Additional results
Obon-Santacana, ³ 2013	Cohort	865	0.77 (0.58–1.04)	0.95 (0.89–1.01)	No heterogeneity by smoking and sex. Inverse association in obese subjects
Larynx					
Pelucchi, ³³ 2006	Case-control	527	1.23 (0.80–1.90)	1.03 (0.96–1.12) ¹	
Schouten, ¹⁶ 2009	Case-cohort	36	0.93 (0.54–1.58)	1.05 (0.91–1.21)	No association in men nor in nonsmokers
Lung					
Hogervorst, ²² 2009, men	Case-cohort	1600	1.03 (0.77–1.39)	1.03 (0.96–1.11)	No heterogeneity by age, smoking, BMI, diabetes, nonoccupational physical activity, alcohol. Similar results for different histologies
Hogervorst, ²² 2009, women	Case-cohort	295	0.45 (0.27–0.76)	0.82 (0.69–0.96)	Lower HRs for intermediate levels of non-occupational physical activity. No heterogeneity by age, smoking, BMI, diabetes, alcohol. Similar results for different histologies
Hirvonen, ⁹ 2010	Cohort	1703	1.18 (1.01–1.38)	1.02 (0.99–1.05) ¹	
Breast					
Mucci, ³¹ 2005	Cohort	667	1.19 (0.91–1.55)	1.01 (0.97–1.06) ¹	
Pelucchi, ³³ 2006	Case-control	2900	1.06 (0.88–1.28)	1.01 (0.98–1.05) ¹	
Hogervorst, ¹⁹ 2007 ⁶	Case-cohort	1350	0.93 (0.73–1.19)	0.99 (0.92–1.06)	No heterogeneity by smoking
Larsson, ²⁶ 2009	Cohort	2952	0.91 (0.80–1.02)	0.98 (0.95–1.01) ¹	No heterogeneity by hormonal receptor status, smoking
Wilson, ³⁵ 2009	Cohort	1179	0.92 (0.76–1.11)	0.98 (0.95–1.02) ¹	No heterogeneity by hormonal receptor status, smoking, age, BMI, alcohol, glycemic index and glycemic load
Burley, ⁸ 2010	Cohort	1084	1.16 (0.88–1.52)	1.08 (0.98–1.18)	Positive association in premenopausal women. No association in never smokers
Pedersen, ¹⁰ 2010	Case-cohort	1690	0.92 (0.73–1.15)	0.97 (0.91–1.03)	No significant association emerged by ER and PR status, and in never smokers, though risks were somewhat increased in never smoking ER+, PR+ and ER+/PR+ cases
Wilson, ⁵ 2010	Cohort	6301	0.95 (0.87–1.03)	0.99 (0.96–1.02) ¹	Similar results in never smokers and according to ER/PR status. No heterogeneity by menopausal status and BMI
Endometrium					
Hogervorst, ¹⁹ 2007	Case-cohort	221	1.29 (0.81–2.07)	1.04 (0.91–1.19)	Higher risk in nonsmokers
Larsson, ²⁸ 2009	Cohort	687	0.96 (0.76–1.21)	1.01 (0.94–1.07) ¹	No heterogeneity by smoking, menopausal status
Wilson, ⁵ 2010	Cohort	484	1.41 (1.01–1.97)	1.15 (1.02–1.31) ¹	Similar results in never smokers. No heterogeneity by menopausal status and BMI, though risks were somewhat higher in those with BMI <25 kg/m ²
Obon-Santacana, ⁴ 2014	Cohort	1382	0.98 (0.78–1.25)	0.98 (0.92–1.05)	No heterogeneity by smoking, alcohol, BMI. Decreased risk of type-I endometrial cancer only in OC users, and in those with BMI <25 kg/m ² . Increased risk of type-I endometrial cancer only in never smokers who never used OC, and decreased risk in ever smokers who ever used OC

Table 1. RR and corresponding 95% CI of specific cancer sites, for high versus low level and for an increase of 10 µg/day of dietary acrylamide intake (Continued)

Cancer site; first author, reference, year	Study design	No. cases	High vs. low level of intake RR (95% CI)	10 µg/day increase in intake RR (95% CI)	Additional results
Ovary					
Pelucchi, ³³ 2006	Case-control	1031	0.97 (0.73–1.31)	0.99 (0.94–1.05) ¹	
Hogervorst, ¹⁹ 2007	Case-cohort	195	1.78 (1.10–2.88)	1.11 (0.99–1.25)	Higher risk in nonsmokers
Larsson, ²⁵ 2009	Cohort	368	0.86 (0.63–1.16)	0.97 (0.89–1.05) ¹	No heterogeneity by smoking, alcohol, HRT, OC
Wilson, ⁵ 2010	Cohort	416	1.25 (0.88–1.77)	1.10 (0.97–1.25) ¹	Similar results in never smokers. No heterogeneity by menopausal status and BMI, though risks were somewhat higher in those with BMI <25 kg/m ² . Higher risk for serous cancers
Prostate					
Pelucchi, ³³ 2006	Case-control	1294	0.92 (0.69–1.23)	0.98 (0.93–1.04) ¹	
Hogervorst, ²¹ 2008	Case-cohort	2246	1.06 (0.87–1.30)	1.01 (0.96–1.07)	No heterogeneity by smoking, alcohol, diabetes, physical activity
Larsson, ²⁷ 2009	Cohort	2696	0.88 (0.70–1.09)	0.98 (0.94–1.02) ¹	No heterogeneity by smoking
Wilson, ³⁴ 2009	Case-control	1499	0.97 (0.75–1.27)	0.99 (0.92–1.06)	No heterogeneity by smoking
Hirvonen, ⁹ 2010	Cohort	799	1.05 (0.83–1.32)	1.01 (0.97–1.05) ¹	
Wilson, ¹¹ 2012	Cohort	5025	1.02 (0.92–1.13)	1.01 (0.99–1.03) ¹	Similar results in never smokers, and in those with lethal, advanced, localized, high grade and low grade disease
Bladder					
Mucci, ^{17,30} 2003 ^{3,4}	Case-control	263	0.89 (0.5–1.6)	1.00 (0.92–1.08) ¹	No heterogeneity by smoking
Hogervorst, ²¹ 2008	Case-cohort	1210	0.91 (0.73–1.15)	1.00 (0.95–1.06)	No heterogeneity by alcohol, diabetes, physical activity. Lower risk in nonsmokers
Hirvonen, ⁹ 2010	Cohort	365	0.99 (0.71–1.39)	0.99 (0.93–1.05) ¹	
Kidney					
Mucci, ^{17,30} 2003 ^{3,4}	Case-control	133	0.65 (0.4–1.3)	0.91 (0.84–1.00) ¹	No heterogeneity by smoking
Mucci, ¹⁸ 2004 ^{3,4}	Case-control	379	1.1 (0.7–1.8)	1.03 (0.94–1.12) ¹	No heterogeneity by smoking
Pelucchi, ³² 2007	Case-control	767	1.20 (0.88–1.63)	1.03 (0.98–1.08) ¹	No heterogeneity by sex, age
Hogervorst, ²¹ 2008	Case-cohort	339	1.59 (1.09–2.30)	1.10 (1.01–1.21)	No heterogeneity by smoking, alcohol, diabetes, physical activity
Hirvonen, ⁹ 2010	Cohort	184	1.28 (0.76–2.15)	1.07 (0.98–1.17) ¹	
Brain					
Hogervorst, ²³ 2009	Case-cohort	216	0.87 (0.54–1.41)	1.02 (0.89–1.16)	No heterogeneity by smoking. Similar results for astrocytic glioma
Thyroid					
Schouten, ¹⁶ 2009	Case-cohort	25	1.33 (0.70–2.53)	1.03 (0.86–1.24)	No association in women nor in nonsmokers
Lymphoid malignancies					
Hirvonen, ⁹ 2010 (lymphoma)	Cohort	175	1.10 (0.67–1.80)	1.02 (0.93–1.11) ¹	
Bongers, ⁷ 2012 (MM, men) ⁷	Case-cohort	170	1.54 (0.92–2.58)	1.14 (1.01–1.27)	Higher risk in never smokers

Table 1. RR and corresponding 95% CI of specific cancer sites, for high versus low level and for an increase of 10 µg/day of dietary acrylamide intake (Continued)

Cancer site; first author, reference, year	Study design	No. cases	High vs. low level of intake RR (95% CI)	10 µg/day increase in intake RR (95% CI)	Additional results
Bongers, ⁷ 2012 (MM, women) ⁷	Case-cohort	153	0.93 (0.50–1.73)	0.92 (0.77–1.11)	No heterogeneity by smoking
Bongers, ⁷ 2012 (DLCL, men) ⁷	Case-cohort	159	1.06 (0.61–1.86)	1.04 (0.91–1.20)	No heterogeneity by smoking
Bongers, ⁷ 2012 (DLCL, women) ⁷	Case-cohort	100	1.38 (0.63–3.02)	1.02 (0.85–1.24)	No heterogeneity by smoking
Bongers, ⁷ 2012 (CLL, men) ⁷	Case-cohort	134	NA	0.88 (0.74–1.04)	No heterogeneity by smoking
Bongers, ⁷ 2012 (CLL, women) ⁷	Case-cohort	66	0.81 (0.42–1.57)	0.83 (0.64–1.09)	No heterogeneity by smoking
Bongers, ⁷ 2012 (follicular lymphoma, men) ⁷	Case-cohort	42	NA	1.28 (1.03–1.61)	
Bongers, ⁷ 2012 (follicular lymphoma, women) ⁷	Case-cohort	47	NA	1.12 (0.80–1.57)	
Bongers, ⁷ 2012 (WMI, men) ⁷	Case-cohort	54	NA	1.18 (0.93–1.50)	
Bongers, ⁷ 2012 (WMI, women) ⁷	Case-cohort	35	NA	1.21 (0.88–1.66)	
Bongers, ⁷ 2012 (mantle cell lymphoma, only men) ⁷	Case-cohort	38	NA	1.06 (0.85–1.31)	
Bongers, ⁷ 2012 (T-cell lymphoma, only men) ⁷	Case-cohort	35	NA	0.94 (0.68–1.29)	

BMI: body mass index; CLL: chronic lymphocytic leukemia; DLCL: diffuse large cell lymphoma; ER: estrogen-receptor; HR: hazard ratio; HRT: hormone replacement therapy; MM: multiple myeloma; NA: not available; OC: oral contraceptives; OR: odds ratio; PR: progesterone receptor; SCC: squamous cell carcinoma; WMI: Waldenstrom macroglobulinemia and immunocytoma.

¹Estimated using the method proposed by Greenland and Longnecker.³⁶

²When we pooled the estimates from Schouten et al, 2009, the RRs of all oral cavity and pharynx cancers were 0.66 (95% CI, 0.42–1.04) for high versus low intake and 0.85 (95% CI, 0.72–1.02) for continuous exposure. Both pooled estimates were obtained using a fixed-effect meta-analysis. These estimates were included in the meta-analysis of Table 2.

³Mean acrylamide intake of open-ended quartiles was estimated using data from other Swedish studies on dietary acrylamide.

⁴The exact RR estimates of Ref. 17 for colorectal and bladder cancer and of Ref. 18 for kidney cancer were provided by Prof. Mucci (personal communication), while the other estimates and the 95% CI were no longer available and were thus derived from Figure 2 of Ref. 17 and from Figure 1 of Ref. 18.

⁵Estimates were not included in the meta-analysis since the data derived from the same prospective investigation of Hogervorst et al., 2008²⁰, and the latter was based on a larger number of cases and a longer follow-up period.

⁶Estimates were not included in the meta-analysis since the data derived from the same prospective investigation of Pedersen et al., 2010¹⁰, and the latter had an extended follow-up period.

⁷When we pooled the available estimates from Bongers et al, 2012, the RRs of all lymphoid malignancies were 1.30 (95% CI, 0.89–1.89) for high versus low intake and 1.07 (95% CI, 1.00–1.15) for continuous exposure in men, and 0.98 (95% CI, 0.66–1.44) for high versus low intake and 0.98 (95% CI, 0.88–1.09) for continuous exposure in women. Pooled estimates were obtained using the Hamling method⁴⁰ for high versus low exposure and a fixed-effect meta-analysis for continuous exposure. These estimates were included in the meta-analysis of Table 2.

menopausal status and hormone receptor status. The pooled RRs for high versus low acrylamide intake from cohort studies only were 1.01 (95% CI, 0.71–1.43) for esophageal, 0.96 (95% CI, 0.85–1.09) for colorectal, 0.87 (95% CI, 0.71–1.07) for pancreatic, 0.96 (95% CI, 0.90–1.01) for breast, 1.06 (95% CI, 0.92–1.23) for endometrial, 1.20 (95% CI, 0.81–1.79) for ovarian, 1.01 (95% CI, 0.93–1.09) for prostate and 1.48 (95%

CI, 1.09–2.00) for kidney cancer. The risk of endometrial (RR = 1.23; 95% CI, 1.00–1.51) and ovarian cancer (RR = 1.39; 95% CI, 0.97–2.00) for high acrylamide intake was of borderline significance in the subgroup of never smoking women. For kidney cancer, the RRs for high versus low acrylamide intake were not materially different in never/former smokers (RR = 1.10; 95% CI, 0.63–1.92) and in

Table 2. Results of meta-analyses of epidemiological studies of dietary acrylamide intake and cancer risk.

Cancer site	No. of estimates	Total no. of cases	High vs. low level of intake		10 µg/day increase in intake	
			RR (95% CI) ¹	p for heterogeneity	RR (95% CI) ¹	p for heterogeneity
Oral cavity and pharynx	2	933	0.87 (0.52–1.46)	0.08	0.95 (0.80–1.11)	0.07
Esophagus	4	1546	1.14 (0.93–1.38)	0.41	1.03 (0.99–1.07)	0.43
Stomach	2	787	1.03 (0.79–1.33)	0.73	1.01 (0.96–1.07)	0.86
Colorectum	6	6794	0.94 (0.85–1.04)	0.65	1.00 (0.98–1.01)	0.14
Pancreas	4	1732	0.93 (0.76–1.12)	0.24	0.99 (0.95–1.03)	0.28
Larynx	2	707	1.10 (0.79–1.54)	0.43	1.03 (0.97–1.11)	0.82
Lung	3 ²	3598	0.88 (0.57–1.34)	0.002	0.99 (0.91–1.07)	0.04
Breast	7	16,773	0.96 (0.91–1.02)	0.37	1.00 (0.98–1.01)	0.33
Endometrium	4	2774	1.06 (0.92–1.23)	0.20	1.01 (0.97–1.06)	0.17
Ovary	4	2010	1.12 (0.85–1.47)	0.06	1.01 (0.97–1.05)	0.12
Prostate	6	13,559	1.00 (0.93–1.08)	0.81	1.00 (0.99–1.02)	0.74
Bladder	3	1838	0.93 (0.78–1.11)	0.91	1.00 (0.96–1.03)	0.97
Kidney	5	1802	1.20 (1.00–1.45)	0.16	1.02 (0.97–1.08)	0.03
Lymphoid malignancies	3 ²	1208	1.13 (0.89–1.43)	0.59	1.03 (0.99–1.09)	0.37

RR: relative risk; CI: confidence interval

¹RR from random-effects model when p for heterogeneity ≤0.10, and from fixed-effects model elsewhere.²Based on three estimates from two studies, since for one study the RRs were given separately for men and women.

current smokers (RR = 1.28; 95% CI, 0.78–2.11). No difference in risk of breast cancer with high acrylamide intake emerged according to menopausal status nor for different hormone receptor cancer types.

Discussion

This updated quantitative review of the available evidence on dietary acrylamide and cancer risk confirms previous indications of a lack of association with most cancer sites, as well as of a potential small increase in the risk of kidney cancer, and of endometrial and ovarian cancer in never smoking women, at high levels of acrylamide intake. The findings reported by different studies were generally not heterogeneous, and results were confirmed when only cohort investigation were retained in the analyses. The total number of cancer cases included in this updated meta-analysis was approximately doubled by including the new data released over the last 5 years, mainly from large prospective cohort studies.^{2–5,7–9,11} Thus, we were able to examine a higher number of cancer sites, as well as to achieve an increased statistical power – with, therefore, narrower CIs for several estimates. This notwithstanding, for some cancer sites the number of studies is still limited.

As concerns kidney cancer, we reported a 20% increase in risk in subjects with high as compared to low dietary acrylamide intake, based on data from three case-control and two cohort studies. Considering only the latter study design, the increase was somewhat higher, that is, about 50%. On the other hand, there was no significant association when the

analyses were conducted using a continuous measure of exposure (+2% in risk for an increase of 10 µg/day of dietary acrylamide). The latter analysis was however affected by relevant heterogeneity between study estimates, ranging from 0.91 in a Swedish case-control investigation^{17,30} to 1.10 in the NCS.²¹ This may be at least in part explained by different results according to study design with, again, cohort studies reporting moderately higher risk estimates.^{9,21} Smoking is a major risk factor for kidney cancer⁴¹ and a major source of acrylamide exposure, too.⁴² Thus, smoking might have a confounding or modifying effect on the relation between dietary acrylamide and kidney cancer. All the five studies included in the meta-analysis of kidney cancer, however, controlled their risk estimates for some measure of tobacco smoking, and when we performed subgroup meta-analyses according to smoking status, we found similar RRs among never/former and current smokers. Limited indications for a potential role of acrylamide exposure on cancer of the kidney were also provided from two occupational cohort studies.^{43,44} Given the modest, borderline significant, association and the still limited amount of epidemiological data on kidney cancer (*i.e.*, about 1,800 cases), the issue remains therefore open to discussion.

The main pathway to carcinogenesis of acrylamide is through its oxidization to glycidamide, a chemically reactive genotoxic metabolite.⁴⁵ Besides damaging and mutagenic effects on DNA, the existence of other modes of action of acrylamide is supported by the observation of a tissue-specific cancerogenicity in both mice and rats.⁴⁶ Acrylamide

Table 3. Meta-analysis of high versus low dietary acrylamide intake in relation to the risk of selected cancers, in subgroups of study design and selected covariates.

Cancer site/subgroup	No. of studies	RR (95% CI) ¹
Esophagus		
Cohort studies ²	2	1.01 (0.71–1.43)
Case-control studies	2	1.20 (0.94–1.53)
Never/former smokers	3	1.27 (0.91–1.77)
Colorectum		
Cohort studies ²	4	0.96 (0.85–1.09)
Case-control studies	2	0.91 (0.76–1.08)
Never/former smokers	3	0.97 (0.79–1.18)
Current smokers	3	0.98 (0.74–1.29)
Pancreas		
Cohort studies ²	3	0.87 (0.71–1.07)
Case-control studies	1	1.49 (0.83–2.70)
Breast		
Cohort studies ²	6	0.96 (0.90–1.01)
Case-control studies	1	1.06 (0.88–1.28)
Never smokers	5	0.91 (0.83–1.01)
Ever smokers	2	0.98 (0.78–1.24)
Premenopausal women	3	1.02 (0.89–1.17)
Postmenopausal women	3	0.93 (0.85–1.02)
ER+PR+ cancer	4	0.98 (0.89–1.08)
ER+PR- cancer	2	1.09 (0.89–1.33)
ER-PR+ cancer	1	1.09 (0.63–1.87)
ER-PR- cancer	4	0.89 (0.75–1.06)
Endometrium ³		
Never smokers	4	1.23 (1.00–1.51)
Ever smokers	2	0.91 (0.67–1.23)
Ovary		
Cohort studies ²	3	1.20 (0.81–1.79) ⁴
Case-control studies	1	0.97 (0.73–1.31)
Never smokers	3	1.39 (0.97–2.00)
Ever smokers	1	1.42 (0.71–2.82)
Prostate		
Cohort studies ²	4	1.01 (0.93–1.09)
Case-control studies	2	0.95 (0.78–1.15)
Never smokers	3	0.95 (0.84–1.08)
Kidney		
Cohort studies ²	2	1.48 (1.09–2.00)
Case-control studies	3	1.06 (0.84–1.35)
Never/former smokers	2	1.10 (0.63–1.92)
Current smokers	2	1.28 (0.78–2.11)

CI: confidence interval; ER: estrogen-receptor; PR: progesterone receptor; RR: relative risk

¹RR from random-effects model when p for heterogeneity ≤ 0.10 , and from fixed-effects model elsewhere.

²Including case-cohort designs.

³Analyses by study design are not presented since all the studies were cohorts.

⁴ p for heterogeneity within subgroup ≤ 0.10 .

may also exert a carcinogenic role on selected body sites by affecting hormonal balances.⁴⁷ This, together with the findings of animal studies that reported an increased occurrence of mammary gland tumors in rats which were given acrylamide through drinking water,^{48,49} explains the wide interest in investigating the relation with breast, but also endometrial and ovarian, cancer in epidemiological studies.

Our meta-analysis, however, did not report an association with breast cancer risk. In particular, the summary RRs of dietary acrylamide and breast cancer were below unity for high versus low intake, as well as in subgroups of prospective studies, of both never and ever smokers, and of both estrogen receptor positive (ER+)/progesterone receptor positive (PR+) and ER-PR- breast cancers. Two other publications (both from the Danish, Diet, Cancer and Health study)^{42,50} considered acrylamide exposure in relation to breast cancer, but were not included in the present meta-analysis, because: (i) the first investigation considered biomarkers of exposure, that is, acrylamide-hemoglobin (Hb) and glycidamide-Hb adducts, but not dietary intake.⁴² This study found a positive association between acrylamide-Hb level and ER+ (RR = 2.7; 95% CI, 1.1–6.6, for a 10-fold increase in adduct concentrations) but not ER- breast cancer, the overall RR being 1.9 (95% CI, 0.9–4.0). Results for ER+ breast cancer were lower in nonsmokers (RR = 1.9) than in smokers (RR = 4.9), and a significant increase in breast cancer risk emerged only after careful adjustment for smoking habits, including amount, duration and past smoking. Smokers had over threefold higher levels of acrylamide adducts than nonsmokers, in both cases and controls. The lack of matching of cases and controls on smoking status—which had an important role in that study—makes it, therefore, difficult to interpret the results; (ii) the second investigation analyzed the role of pre-diagnostic biomarkers of acrylamide exposure on survival after a diagnosis of breast cancer.⁵⁰ Breast cancer specific mortality increased with increasing adduct levels, particularly in nonsmoking women with ER+ cancer (HR = 1.31, 95% CI, 1.02–1.69, for a 25 pmol/g globin increase in acrylamide-Hb; HR = 2.23, 95% CI, 1.38–3.61, for a 25 pmol/g globin increase in glycidamide-Hb level). Thus, the results on biomarkers of acrylamide exposure of the Danish, Diet, Cancer and Health study^{42,50} indicated significant increases of both ER+ breast cancer risk and mortality, that should be further investigated. This meta-analysis of data on dietary acrylamide, on the other hand, did not find associations with ER+PR+ nor with ER+PR- breast cancer risk.

The role of acrylamide on endometrial and ovarian cancers has been the object of a recent debate.^{51–53} In this updated review, we were able to include additional data from the NHS and EPIC (for endometrial cancer only) studies.^{4,5} Still, summary results were close to those of our previous report confirming, overall, an absence of association, but a moderate increase in risk in the subgroup of never smoking women. For ovarian cancer, some caution is needed in the interpretation of results, given the unexplained heterogeneity

between estimates. Another study examined data of the NHS and NHS II on ovarian cancer risk according to Hb adduct levels, and was thus not included in the meta-analysis on dietary acrylamide.⁶ This reported no association in the complete dataset (RR = 0.79; 95% CI, 0.50–1.24 for highest versus lowest tertile of combined acrylamide-Hb and glycidamide-Hb adducts) nor in the subgroup of nonsmoking women (RR = 0.84; 95% CI, 0.55–1.27). Overall, epidemiological studies published to date do not support an association between dietary acrylamide intake and major female hormone-related cancers. Associations in specific subgroups, particularly among never smokers for endometrial and ovarian cancers, cannot be excluded,⁴ and need further investigation.

With reference to other cancer sites, a considerable amount of data is now available on the role of dietary acrylamide on colorectal and prostate cancers. Overall results do not indicate any excess risk for these cancer sites. For colorectum, however, a recent study reported for the first time differential results for acrylamide and colorectal cancer with specific somatic mutations, with a more than twofold increased risk of cancers with an activating Kirsten-ras (KRAS) mutation in men and a halved risk of cancers with a truncating adenomatous polyposis coli (APC) mutation in women with high versus low acrylamide intake.¹³ To date, specific information on the molecular characteristics of colorectal tumors in relation to acrylamide intake is limited to this investigation. For prostate cancer, one of the studies included in the meta-analysis provided further results for acrylamide-Hb adduct levels, besides dietary intake, reporting no association.³⁴ As concerns pancreatic cancer, a possible link with acrylamide exposure has been suggested by occupational cohort studies.^{1,43,44} On the other hand, none of the four epidemiological studies on dietary intake of acrylamide reported any association, and the summary RR of dietary

studies was below unity. While no relation emerged in the meta-analysis of lymphoid malignancies as a whole, a positive association was found, in the only study that considered different types of lymphoid malignancies separately,⁷ with multiple myeloma and with follicular lymphoma in men. For other cancer sites as well, associations were null or weak, although in some cases based on limited data and/or on heterogeneous risk estimates (such as for lung cancer, where heterogeneity was mainly due to different results in men and women in one study²²).

Epidemiological studies on dietary acrylamide have been criticized for their alleged inadequacy to address its relation with cancer risk.^{54–56} Besides general limitations of cohort and case-control studies, we acknowledge several critical aspects related to this specific topic, including the difficulties in estimating dietary acrylamide intake through food frequency questionnaires and databases of acrylamide content in foods, the lack of repeated exposure estimations over an adequately long time-period, and the lack of statistical power to detect small increases in risk. However, epidemiological studies also have several strengths. In particular, they allow to directly address the relation between acrylamide exposure and cancer risk in humans, avoiding the uncertainties deriving from the use of animal data and mathematical models, and to assess the public health relevance of such a relation.⁵⁷ During the last decades, epidemiological studies identified several dietary and nutritional factors associated with the risk of various cancers, particularly those of the digestive tract.⁵⁸ This weighs in favor of the capability of epidemiological studies to assess the association with dietary acrylamide. Further, the meta-analytic approach – in the absence of relevant heterogeneity, as was the case for most of the analyses presented here – increases to a great extent the statistical power of the investigation, by joining together the results of several studies.

References

1. Pelucchi C, La Vecchia C, Bosetti C, et al. Exposure to acrylamide and human cancer—a review and meta-analysis of epidemiologic studies. *Ann Oncol* 2011;22:1487–99.
2. Lujan-Barroso L, Gonzalez CA, Slimani N, et al. Dietary intake of acrylamide and esophageal cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort. *Cancer Causes Control* 2014;25:639–46.
3. Obon-Santacana M, Slimani N, Lujan-Barroso L, et al. Dietary intake of acrylamide and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. *Ann Oncol* 2013;24:2645–51.
4. Obon-Santacana M, Kaaks R, Slimani N, et al. Dietary intake of acrylamide and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort. *Br J Cancer* 2014;111:987–97.
5. Wilson KM, Mucci LA, Rosner BA, Willett WC. A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancers. *Cancer Epidemiol Biomarkers Prev* 2010;19:2503–15.
6. Xie J, Terry KL, Poole EM, et al. Acrylamide hemoglobin adduct levels and ovarian cancer risk: a nested case-control study. *Cancer Epidemiol Biomarkers Prev* 2013;22:653–60.
7. Bongers ML, Hogervorst JG, Schouten LJ, et al. Dietary acrylamide intake and the risk of lymphatic malignancies: the Netherlands Cohort Study on diet and cancer. *PLoS One* 2012;7:e38016.
8. Burley VJ, Greenwood DC, Hepworth SJ, et al. Dietary acrylamide intake and risk of breast cancer in the UK women's cohort. *Br J Cancer* 2010;103:1749–54.
9. Hirvonen T, Kontto J, Jestoi M, et al. Dietary acrylamide intake and the risk of cancer among Finnish male smokers. *Cancer Causes Control* 2010;21:2223–9.
10. Pedersen GS, Hogervorst JG, Schouten LJ, et al. Dietary acrylamide intake and estrogen and progesterone receptor-defined postmenopausal breast cancer risk. *Breast Cancer Res Treat* 2010;122:199–210.
11. Wilson KM, Giovannucci E, Stampfer MJ, Mucci LA. Dietary acrylamide and risk of prostate cancer. *Int J Cancer* 2012;131:479–87.
12. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000;283:2008–12.
13. Hogervorst JG, de Bruijn-Geraets D, Schouten LJ, et al. Dietary acrylamide intake and the risk of colorectal cancer with specific mutations in KRAS and APC. *Carcinogenesis* 2014;35:1032–8.
14. Lin Y, Lagergren J, Lu Y. Dietary acrylamide intake and risk of esophageal cancer in a population-based case-control study in Sweden. *Int J Cancer* 2011;128:676–81.
15. Pelucchi C, Galeone C, Talamini R, et al. Dietary acrylamide and pancreatic cancer risk in an Italian case-control study. *Ann Oncol* 2011;22:1910–5.

16. Schouten LJ, Hogervorst JG, Konings EJ, et al. Dietary acrylamide intake and the risk of head-neck and thyroid cancers: results from the Netherlands Cohort Study. *Am J Epidemiol* 2009;170:873–84.
17. Mucci LA, Dickman PW, Steineck G, et al. Reply: Dietary acrylamide and cancer risk: additional data on coffee. *Br J Cancer* 2003;89:775–76.
18. Mucci LA, Lindblad P, Steineck G, Adami HO. Dietary acrylamide and risk of renal cell cancer. *Int J Cancer* 2004;109:774–6.
19. Hogervorst JG, Schouten LJ, Konings EJ, et al. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev* 2007;16:2304–13.
20. Hogervorst JG, Schouten LJ, Konings EJ, et al. Dietary acrylamide intake is not associated with gastrointestinal cancer risk. *J Nutr* 2008;138:2229–36.
21. Hogervorst JG, Schouten LJ, Konings EJ, et al. Dietary acrylamide intake and the risk of renal cell, bladder, and prostate cancer. *Am J Clin Nutr* 2008;87:1428–38.
22. Hogervorst JG, Schouten LJ, Konings EJ, et al. Lung cancer risk in relation to dietary acrylamide intake. *J Natl Cancer Inst* 2009;101:651–62.
23. Hogervorst JG, Schouten LJ, Konings EJ, et al. Dietary acrylamide intake and brain cancer risk. *Cancer Epidemiol Biomarkers Prev* 2009;18:1663–6.
24. Larsson SC, Akesson A, Bergkvist L, Wolk A. Dietary acrylamide intake and risk of colorectal cancer in a prospective cohort of men. *Eur J Cancer* 2009;45:513–6.
25. Larsson SC, Akesson A, Wolk A. Long-term dietary acrylamide intake and risk of epithelial ovarian cancer in a prospective cohort of Swedish women. *Cancer Epidemiol Biomarkers Prev* 2009;18:994–7.
26. Larsson SC, Akesson A, Wolk A. Long-term dietary acrylamide intake and breast cancer risk in a prospective cohort of Swedish women. *Am J Epidemiol* 2009;169:376–81.
27. Larsson SC, Akesson A, Wolk A. Dietary acrylamide intake and prostate cancer risk in a prospective cohort of Swedish men. *Cancer Epidemiol Biomarkers Prev* 2009;18:1939–41.
28. Larsson SC, Hakansson N, Akesson A, Wolk A. Long-term dietary acrylamide intake and risk of endometrial cancer in a prospective cohort of Swedish women. *Int J Cancer* 2009;124:1196–9.
29. Mucci LA, Adami HO, Wolk A. Prospective study of dietary acrylamide and risk of colorectal cancer among women. *Int J Cancer* 2006;118:169–73.
30. Mucci LA, Dickman PW, Steineck G, et al. Dietary acrylamide and cancer of the large bowel, kidney, and bladder: absence of an association in a population-based study in Sweden. *Br J Cancer* 2003;88:84–9.
31. Mucci LA, Sandin S, Balter K, et al. Acrylamide intake and breast cancer risk in Swedish women. *JAMA* 2005;293:1326–7.
32. Pelucchi C, Galeone C, Dal Maso L, et al. Dietary acrylamide and renal cell cancer. *Int J Cancer* 2007;120:1376–7.
33. Pelucchi C, Galeone C, Levi F, et al. Dietary acrylamide and human cancer. *Int J Cancer* 2006;118:467–71.
34. Wilson KM, Balter K, Adami HO, et al. Acrylamide exposure measured by food frequency questionnaire and hemoglobin adduct levels and prostate cancer risk in the Cancer of the Prostate in Sweden Study. *Int J Cancer* 2009;124:2384–90.
35. Wilson KM, Mucci LA, Cho E, et al. Dietary acrylamide intake and risk of premenopausal breast cancer. *Am J Epidemiol* 2009;169:954–61.
36. Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol* 1992;135:1301–9.
37. Orsini N, Bellocco R, Greenland S. Generalized least squares for trend estimation of summarized dose-response data. *Stat J* 2006;6:40–57.
38. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;7:177–88.
39. Greenland S. Quantitative methods in the review of epidemiologic literature. *Epidemiol Rev* 1987;9:1–30.
40. Hamling J, Lee P, Weitkunat R, Ambuhl M. Facilitating meta-analyses by deriving relative effect and precision estimates for alternative comparisons from a set of estimates presented by exposure level or disease category. *Stat Med* 2008;27:954–70.
41. McLaughlin JK, Lipworth L, Tarone RE, Blot WJ. Renal cancer. In: Schottenfeld D, Fraumeni JF. *Cancer epidemiology and prevention*, 3rd ed. New York: Oxford University Press, 2006. 1087–100.
42. Olesen PT, Olsen A, Frandsen H, et al. Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study. *Int J Cancer* 2008;122:2094–100.
43. Marsh GM, Youk AO, Buchanich JM, et al. Mortality patterns among workers exposed to acrylamide: updated follow up. *J Occup Environ Med* 2007;49:82–95.
44. Swaen GM, Haidar S, Burns CJ, et al. Mortality study update of acrylamide workers. *Occup Environ Med* 2007;64:396–401.
45. International Agency for Research on Cancer. Some industrial chemicals. Vol. 60. Lyon: International Agency for Research on Cancer, 1994.
46. Besaratinia A, Pfeifer GP. A review of mechanisms of acrylamide carcinogenicity. *Carcinogenesis* 2007;28:519–28.
47. Hogervorst JG, Baars BJ, Schouten LJ, et al. The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. *Crit Rev Toxicol* 2010;40:485–512.
48. Johnson KA, Gorzinski SJ, Bodner KM, et al. Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol Appl Pharmacol* 1986;85:154–68.
49. Rice JM. The carcinogenicity of acrylamide. *Mutat Res* 2005;580:3–20.
50. Olsen A, Christensen J, Outzen M, et al. Pre-diagnostic acrylamide exposure and survival after breast cancer among postmenopausal Danish women. *Toxicology* 2012;296:67–72.
51. Lipworth L, Sonderman JS, Tarone RE, McLaughlin JK. Review of epidemiologic studies of dietary acrylamide intake and the risk of cancer. *Eur J Cancer Prev* 2012;21:375–86.
52. Hogervorst J, Duell E, Schouten L, et al. Reaction on the acrylamide and cancer review by Lipworth and colleagues. *Eur J Cancer Prev* 2013;22:194–8.
53. Lipworth L, Sonderman JS, Tarone RE, McLaughlin JK. Acrylamide: a human cancer risk? *Eur J Cancer Prev* 2013;22:193–4.
54. Granath F, Tornqvist M. Who knows whether acrylamide in food is hazardous to humans? *J Natl Cancer Inst* 2003;95:842–3.
55. Hagmar L, Tornqvist M. Inconclusive results from an epidemiological study on dietary acrylamide and cancer. *Br J Cancer* 2003;89:774–5; author reply 75–6.
56. Virk-Baker MK, Nagy TR, Barnes S, Groopman J. Dietary acrylamide and human cancer: a systematic review of literature. *Nutr Cancer* 2014;66:774–90.
57. Mucci LA, Wilson KM. Acrylamide intake through diet and human cancer risk. *J Agric Food Chem* 2008;56:6013–9.
58. American Institute for Cancer Research. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research, 2007.

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Dietary Acrylamide and the Risk of Endometrial Cancer: An Italian Case-Control Study

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ABSTRACT

The role of dietary acrylamide on the risk of hormone-related, and specifically endometrial, cancers is debated. Epidemiological data are scanty. Thus, we examined the relation between acrylamide intake and endometrial cancer risk in a case-control study conducted between 1992 and 2006 in 3 Italian areas. Cases were 454 women with incident, histologically confirmed endometrial cancer. Controls were 908 age-matched women admitted to the same network of hospitals of cases for acute, non-neoplastic conditions. We calculated multivariate odds ratios (OR) and 95% confidence intervals (CI) using logistic regression models. The OR of endometrial cancer for increasing quintiles of dietary acrylamide, as compared to the lowest one, were 1.02 (95% CI: 0.67–1.54), 1.20 (95% CI: 0.80–1.80), 1.00 (95% CI: 0.65–1.54) and 1.17 (95% CI: 0.73–1.85). The OR for an increase of 10 $\mu\text{g}/\text{day}$ of dietary acrylamide was 1.00 (95% CI: 0.91–1.10). In subgroup analyses, the ORs for high vs. low acrylamide intake were 1.28 (95% CI: 0.73–2.25) in never smokers and 1.14 (95% CI: 0.45–2.90) in ever smokers. Our data do not support an association between dietary acrylamide intake and endometrial cancer.

ARTICLE HISTORY

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Introduction

Acrylamide is a chemical classified as a possible human carcinogen by the International Agency for Research on Cancer (1). The potential association between dietary acrylamide and (increased) cancer risk has been debated for over a decade now (2,3)—after Swedish researchers reported the presence of acrylamide in several foods cooked at high temperatures (4)—and is still controversial (5–8).

In particular, the role of acrylamide on hormone-related cancers, mainly ovarian and endometrial neoplasms, has been debated. This stemmed from the publication of a few papers reporting positive findings (9–11) and the suggestion that—besides a genotoxic effect through its conversion to glycidamide—acrylamide may influence the sex hormone system (11–13).

In a meta-analysis of epidemiological studies published up to June 2009 (14), we assessed the relation between dietary and occupational exposure to acrylamide and cancer risk. No increased risk emerged for most cancer sites except, possibly, kidney cancer. However, only 2 studies were available at that time for endometrial

cancer (10,15). Findings from the Nurses' Health Study, suggesting an increased risk of endometrial cancer in women with high acrylamide intake (9) and from the European Prospective Investigation into Cancer and Nutrition (EPIC), reporting no overall association (16), were published thereafter and were included in a subsequent quantitative meta-analysis (17). This concluded that, overall, dietary acrylamide is not associated to endometrial cancer, though a modest association in never smoking women was not excluded.

Using data from our network of case-control studies, we already examined the relation between dietary acrylamide and the risk of breast and ovarian (as well as other) cancers (18). With the aim of providing further data to the ongoing debate, we considered the association with endometrial cancer in our network of Italian case-control studies.

Patients and methods

Data were derived from a case-control study of endometrial cancer conducted between 1992 and 2006 in 3

Italian areas: the provinces of Pordenone and Milan, in northern Italy, and of Naples, in southern Italy (19).

Cases were 454 women (median age 60 years, range 18–79) with incident, histologically confirmed endometrial cancer, and no previous diagnosis of cancer. Controls were 908 women (median age 61 years, range 19–79) admitted to the same network of hospitals of cases for a wide spectrum of non-neoplastic acute illnesses. Women admitted for gynecological or hormone-related conditions, or any medical condition associated with long-term dietary changes were not eligible as controls. Women with a history of hysterectomy were excluded from the control group. Controls were admitted for traumas (36%), other orthopedic disorders (32%), acute surgical conditions (9%), and miscellaneous other illnesses, including eye, nose, ear, skin or dental disorders (23%). Controls were frequency-matched to cases on age and study center, with a 2:1 ratio. Less than 5% of cases and controls approached refused to be interviewed.

Trained interviewers collected data during the hospital stay of cases and controls, using a standardized, structured questionnaire. This included information on sociodemographic characteristics, anthropometric measures, selected lifestyle habits (e.g., tobacco smoking), personal medical history, family history of cancer, menstrual and reproductive factors, and use of exogenous hormones. A food frequency questionnaire (FFQ) was used to assess the usual diet during the two years before diagnosis (or hospital admission, for controls). The FFQ included 78 foods, food groups or recipes divided into 6 sections: 1) bread, cereals, first courses; 2) second courses (i.e. meat, fish, and other main dishes); 3) side dishes (i.e. vegetables); 4) fruits; 5) sweets, desserts and soft drinks; 6) milk, hot beverages, and sweeteners. At the end of each section, 1 or 2 open-ended questions were used to include other foods eaten at least once per week. An additional section collected data on history of consumption of alcoholic beverages. Subjects were asked to indicate the average weekly frequency of consumption for each dietary item and, for about half of them, their usual portion size. Intakes lower than once a week, but at least once a month, were coded as 0.5 per week. Intake of total energy was computed using an Italian food composition database (20,21). The validity and reproducibility of the FFQ has been previously evaluated, and proved to be satisfactory (22,23).

The items of the FFQ used to estimate daily acrylamide intake were fried/baked potatoes, boiled potatoes, white bread, crackers, breadsticks and melba toast, risotto, pizza, fried meat, fried fish, cookies, beer, regular coffee, decaffeinated coffee, and cappuccino. Data on the average acrylamide content of various foods were obtained from resources available by the World Health

Organization (WHO) and the Agence Francaise de Sécurité Sanitaire des Aliments (24,25). We used specific estimates of acrylamide content of a few Italian foods and beverages, for which data were available (26,27). The correlation coefficient (r) for reproducibility of questions on the main food items related to acrylamide ranged from 0.52 to 0.75 (23).

Odds ratios (OR) and the corresponding 95% confidence intervals (CI) of endometrial cancer according to acrylamide intake were derived using conditional multiple logistic regression (28). All regression models were conditioned age (5-yr age groups, categorically) and study center (Aviano-Pordenone, Milan, Naples), and adjusted for period of interview (1992–1998, 1999–2002, 2003–2006, categorically), education (<7, 7–11, ≥ 12 yr, categorically), body mass index (BMI, <21, 21 to <26, 26 to <30, ≥ 30 kg/m², categorically), history of diabetes (no/yes), occupational physical activity (defined as “heavy/very heavy,” “moderate,” “standing,” or “mainly sitting,” categorically), smoking habit (never smokers, ex-smokers, current smokers of <15, 15–24, ≥ 25 cigarettes/day, categorically), age at menarche (<12, 12–13, 14–15, ≥ 16 years, categorically), menopausal status and age at menopause (pre- and perimenopausal, menopause at <50, 50–52, ≥ 53 yr, categorically), parity (nulliparous, 1, 2, ≥ 3 childbirths, categorically), oral contraceptive (never/ever) and hormone replacement therapy (never/ever) use, and total energy intake (continuously). Analyses across strata of selected covariates (i.e., smoking status, menopausal status, and BMI) were also conducted.

Results

Table 1 shows the distribution of cases and controls, the OR of endometrial cancer and the corresponding 95% CI according to consumption of major contributor foods of total acrylamide intake [i.e., fried/baked potatoes (28%); white bread (24%); cookies (16%); coffee (14%)]; crackers, breadsticks, and melba toast (10%)]. The ORs for intake of fried/baked potatoes were 1.38 (95% CI: 1.03–1.86) for infrequent consumption (i.e., less than 1 portion/wk) and 1.49 (95% CI: 1.06–2.09) for frequent consumption (i.e., 1 or more portions/week), as compared to no consumption. No significant associations emerged for high vs. low or no consumption of white bread (OR = 0.68; 95% CI, 0.46–1.01), cookies (OR = 1.20; 95% CI: 0.80–1.79), coffee (OR = 0.74; 95% CI: 0.53–1.03), and other bakery products (OR = 0.80; 95% CI: 0.59–1.10).

Table 2 reports the distribution of cases and controls, the ORs of endometrial cancer, and their 95% CI, according to quintiles of dietary acrylamide as well as for an intake increase of 10 μ g/day. Stratified results are also given, according to tobacco smoking (never/ever),

Table 1 Frequency distribution of cases and controls, odds ratios (OR) of endometrial cancer and corresponding 95% confidence intervals (CI), according to consumption of foods contributing acrylamide intake: Italy, 1992–2006.

Food item	Cases (n = 454) n (%)	Controls (n = 908) n (%)	OR (95% CI) ¹	% of total acrylamide ²	Mean intake \pm SD ³	
					Cases	Controls
Fried/baked potatoes (portions/week)				28.0%	0.6 \pm 0.6	0.5 \pm 0.7
0	136 (30.0)	357 (39.3)	1			
<1	189 (41.6)	335 (36.9)	1.38 (1.03–1.86)			
≥ 1	129 (28.4)	216 (23.8)	1.49 (1.06–2.09)			
Continuous OR ⁴			1.10 (0.90–1.33)			
White bread (portions/week)				24.2%	12.1 \pm 10.1	12.0 \pm 9.4
≤ 7	210 (46.3)	387 (42.6)	1			
>7–<21	155 (34.1)	352 (38.8)	0.72 (0.54–0.96)			
≥ 21	89 (19.6)	169 (18.6)	0.68 (0.46–1.01)			
Continuous OR ⁵			0.93 (0.84–1.04)			
Cookies (portions/wk)				15.6%	2.3 \pm 3.3	1.9 \pm 2.8
0	166 (36.6)	381 (42.0)	1			
<7	220 (48.5)	413 (45.5)	1.31 (1.00–1.72)			
≥ 7	68 (15.0)	114 (12.6)	1.20 (0.80–1.79)			
Continuous OR ⁵			1.22 (0.91–1.65)			
Coffee (cups/wk) ⁶				14.0%	15.3 \pm 11.3	15.9 \pm 11.0
≤ 7	122 (26.9)	252 (27.8)	1			
>7–<21	182 (40.1)	316 (34.8)	1.20 (0.87–1.65)			
≥ 21	150 (33.0)	340 (37.4)	0.74 (0.53–1.03)			
Continuous OR ⁵			0.91 (0.83–0.99)			
Crackers, breadsticks and melba toast (portions/wk)				9.7%	3.4 \pm 4.4	4.0 \pm 5.5
0	151 (33.3)	304 (33.5)	1			
<7	166 (36.6)	303 (33.4)	1.06 (0.79–1.43)			
≥ 7	137 (30.2)	301 (33.1)	0.80 (0.59–1.10)			
Continuous OR ⁵			0.79 (0.65–0.96)			

¹ ORs from conditional logistic regression models, stratified by study centre and age, and adjusted for period of interview, education, tobacco smoking, body mass index, occupational physical activity, history of diabetes, age at menarche, menopausal status/age at menopause, parity, oral contraceptive use, hormone replacement therapy, and total energy intake.

² Other foods not listed in the Table contributed for 8.5% of total acrylamide intake.

³ The mean intake was expressed in portions/week (or cups/wk).

⁴ OR for an increase of intake of 1 portion/wk.

⁵ OR for an increase of intake of 1 portion/day.

⁶ Including regular and decaffeinated coffee, and cappuccino.

menopausal status (pre- or peri-/postmenopause) and BMI (<25/ \geq 25 kg/m²). The mean intake of dietary acrylamide among controls was 29.8 μ g/day and the standard deviation 16.1 μ g/day. The cut-offs of quintiles of acrylamide intake, computed from the distribution of controls, were 17.7, 24.0, 30.4, and 39.2 μ g/day. The ORs for subsequent quintiles of dietary acrylamide intake, as compared to the lowest one, were 1.02 (95% CI: 0.67–1.54), 1.20 (95% CI: 0.80–1.80), 1.00 (95% CI: 0.65–1.54) and 1.17 (95% CI: 0.73–1.85). No trend in risk was found (P -value = 0.59). The OR for an increase of 10 μ g/day of dietary acrylamide was 1.00 (95% CI: 0.91–1.10). With reference to a possible threshold effect, the OR for comparison of the top 3 vs. the lower 2 quintiles of acrylamide intake (i.e., \geq 24.0 vs. <24.0 μ g/day) was 1.11 (95% CI: 0.84–1.48). Additional adjustment for coffee consumption (excluding decaffeinated coffee, as an indicator of caffeine intake) (9) did not materially change the results. When we examined the relation among subgroups, the ORs for high vs. low acrylamide intake were 1.28 (95% CI: 0.73–2.25) in never smokers and 1.14 (95% CI: 0.45–2.90) in ever smokers; 0.49 (95% CI: 0.14–1.71) in pre- and perimenopausal women and 1.17 (95%

CI, 0.69–1.98) in postmenopausal women; 1.78 (95% CI: 0.82–3.85) in under- and normal weight women and 0.85 (95% CI: 0.46–1.58) in overweight and obese women. In subgroup analyses, no trend in risk emerged, nor significant ORs were found by including a continuous term for acrylamide intake in the models.

Discussion

This large, multicentric case-control study reported no association between dietary acrylamide intake and the risk of endometrial cancer in an Italian population. Among the food items contributing the most to acrylamide intake, a positive association was found with high intake of fried/baked potatoes—that were rather infrequently consumed in our population, but ranked first in terms of acrylamide contribution—whereas white bread and coffee consumption showed borderline inverse relations with endometrial cancer. These findings add to our previous investigations of dietary acrylamide and cancers of the oral cavity and pharynx, esophagus, colorectum, pancreas, larynx, breast, ovary, prostate, and kidney

Table 2 Frequency distribution of cases and controls, odds ratios (OR) ¹ of endometrial cancer and corresponding 95% confidence intervals (CI), according to dietary acrylamide intake overall and in strata of selected covariates²: Italy, 1992–2006.

	Dietary acrylamide intake, OR (95% CI)					χ^2 , trend	Continuous OR ³
	1 st quintile	2 nd quintile	3 rd quintile	4 th quintile	5 th quintile		
All women ⁴							
Cases:controls (454:908)	75:182	79:181	98:182	85:181	117:182		
OR (95% CI)	1 ⁵	1.02 (0.67–1.54)	1.20 (0.80–1.80)	1.00 (0.65–1.54)	1.17 (0.73–1.85)	0.29 (p = 0.59)	1.00 (0.91–1.10)
Never smokers							
Cases:controls (331:646)	56:144	62:128	67:126	57:123	89:125		
OR (95% CI)	1 ⁵	1.21 (0.75–1.95)	1.24 (0.76–2.01)	1.02 (0.60–1.73)	1.28 (0.73–2.25)	0.27 (p = 0.60)	1.02 (0.90–1.15)
Ever smokers							
Cases:controls (123:261)	19:38	17:53	31:56	28:57	28:57		
OR (95% CI)	1 ⁵	0.62 (0.26–1.48)	1.40 (0.62–3.16)	1.18 (0.50–2.77)	1.14 (0.45–2.90)	0.87 (p = 0.35)	1.02 (0.86–1.21)
Pre- and perimenopausal women							
Cases:controls (83:174)	12:15	6:30	19:32	20:49	26:48		
OR (95% CI)	1 ⁵	0.16 (0.04–0.64)	0.51 (0.15–1.65)	0.31 (0.10–0.97)	0.49 (0.14–1.71)	0.10 (p = 0.75)	1.06 (0.86–1.31)
Postmenopausal women							
Cases:controls (360:726)	62:163	70:150	78:147	62:132	88:134		
OR (95% CI)	1 ⁵	1.25 (0.79–1.97)	1.31 (0.84–2.07)	1.06 (0.65–1.75)	1.17 (0.69–1.98)	0.10 (p = 0.75)	0.99 (0.88–1.11)
Body Mass Index <25 kg/m ²							
Cases:controls (131:420)	18:83	17:88	36:75	27:90	33:84		
OR (95% CI)	1 ⁵	1.00 (0.45–2.18)	2.06 (1.01–4.17)	1.38 (0.65–2.91)	1.78 (0.82–3.85)	2.45 (p = 0.12)	1.05 (0.90–1.23)
Body Mass Index ≥25 kg/m ²							
Cases:controls (323:484)	57:98	62:92	62:107	58:90	84:97		
OR (95% CI)	1 ⁵	1.07 (0.64–1.79)	0.84 (0.50–1.41)	0.77 (0.44–1.35)	0.85 (0.46–1.58)	0.89 (p = 0.35)	0.94 (0.83–1.07)

¹ ORs from conditional logistic regression models, conditioned on study centre and age, and adjusted for period of interview, education, tobacco smoking, body mass index, occupational physical activity, history of diabetes, age at menarche, menopausal status/age at menopause, parity, oral contraceptive use, hormone replacement therapy, and total energy intake.

² The sum of subjects do not add up to the total in strata of covariates because of a few missing values.

³ The measurement unit was set at 10 $\mu\text{g/day}$.

⁴ The cut-offs for quintiles of acrylamide intake were 17.7, 24.0, 30.4, and 39.2 $\mu\text{g/day}$.

⁵ Reference category.

(18,29,30), none of them reporting any meaningful association.

In this study, there was no significant association between dietary acrylamide and endometrial cancer in never-smoking women, too. However, the 28% increased risk for high acrylamide intake reported among never smokers is in line with the findings of a recent meta-analysis (17). A potential association in never smokers only remains, therefore, uncertain.

Four other studies, besides ours, provided information on dietary acrylamide and endometrial cancer, with fairly consistent results (9,10,15,16). The Netherlands Cohort Study (10), including 221 cases of endometrial cancer, investigated the role of dietary acrylamide in postmenopausal women and found multivariate relative risks (RR) of 0.95, 0.94, 1.21, and 1.29 for subsequent quintiles of acrylamide intake (P -value for trend = 0.18). Higher risks emerged in never smokers (150 cases), and the corresponding RRs were 1.16, 1.35, 1.30, and 1.99 (the 95% CI for the latter estimate was 1.12–3.52, and the P -value for trend was 0.03). The Swedish

Mammography Cohort study (15), based on 687 incident cases, reported multivariate RRs of 1.10, 1.08, and 0.96 for subsequent quartiles of acrylamide intake, as compared to the lowest one. In a subanalysis based on 273 cases, adjusting for smoking status, the RRs were 1.08, 1.20, and 1.12 in all women and 1.31, 1.30, and 1.20 in never smokers. The Nurses' Health Study (9), including 484 endometrial cancer cases, showed an increased risk for women in the highest quintile of acrylamide intake (RR = 1.41, 95% CI: 1.01–1.97). The corresponding increase in risk was similar in never smokers (RR = 1.43; 95% CI: 0.90–2.28), but somewhat higher in premenopausal (RR = 2.27; 95% CI: 0.96–5.40) and non-overweight women (RR = 2.51; 95% CI: 1.32–4.77). More recent results were provided by the EPIC cohort study, including over 500,000 participants and 1382 cases of endometrial cancer (16). This reported no overall association with dietary acrylamide (hazard ratio, HR = 0.98; 95% CI: 0.78–1.25), while an increased risk of Type I endometrial cancer (i.e., endometrioid adenocarcinomas) emerged in women who never smoked and never

used oral contraceptives (HR = 1.97; 95% CI: 1.08–3.62, for high vs. low acrylamide intake).

We pooled the RRs of the 4 studies plus ours together, thus including a total of 3228 endometrial cancer cases. The summary RR, obtained using a fixed-effect model (31), was 1.07 (95% CI: 0.94–1.23) for the highest vs. lowest level of acrylamide intake. The I^2 for inconsistency between estimates was 16%, indicating that the results of the 5 investigations are relatively homogeneous. The highest estimates were reported by the Nurses' Health Study (9), that showed 2 peculiar characteristics as compared to other investigations; that is, 1) it was based on a population with lower dietary acrylamide levels (as can be derived from the comparison of quintile levels in different studies); 2) it was conducted in the United States, whereas other studies were from Europe. Differences in dietary habits or food preparation (e.g., potatoes fried in olive oil vs. other types of oils, coffee brewing, etc.) between geographic areas may, at least in part, be involved in the explanation of somewhat different findings.

There is some indication of an association between dietary acrylamide and endometrial cancer among subgroups of never smokers (10,14,17) and normal weight women (9). In smokers, acrylamide exposure from tobacco is substantially higher than exposure occurring from dietary sources (32). Because tobacco smoking is also inversely associated to endometrial cancer (33), it is important to analyze separately the relation between dietary acrylamide and endometrial cancer in subgroups of never and ever smokers. In our dataset, however, risks were similar across these strata. With reference to analyses stratified according to BMI, our results were consistent with those of the Nurses' Health Study (9), which suggested a somewhat stronger association with acrylamide intake in the subgroup of normal weight women. However, such an association was not confirmed in the EPIC study, which reported a HR of 0.93 for high acrylamide intake in women with BMI <25 kg/m² (16). Thus, it is likely that modest associations across subgroups are due to the effects of chance.

Potential limitations of this study are those of retrospective investigations, including information and selection bias. However, we asked for the habitual diet during the 2 years before interview or onset of symptoms, and we excluded all the control subjects with diagnoses related to long-term changes in diet or admitted for chronic conditions. Cases and controls were interviewed in the same hospital setting, and dietary information collected in hospital and at home was satisfactorily comparable (34). Further, different selective recall of acrylamide-rich foods in cases and controls is unlikely, as this topic had received little, if any, attention in the

Italian media at the time of data collection. We used information from FFQ and databases of acrylamide content of foods, rather than biomarkers of acrylamide exposure; therefore misclassification of acrylamide exposure is possible. Further, calculation of total acrylamide intake was derived from a relatively small number of FFQ items. However, a valid estimate of dietary acrylamide was previously obtained using a database of mean acrylamide levels of foods (35), and the percent contribution of main foods to total acrylamide intake in our study was comparable with those estimated in other populations with a similar diet (24, 36). Finally, the FFQ used in this study was satisfactorily reproducible for questions on acrylamide-rich items (23).

In conclusion, findings of our case-control study do not support an association between dietary acrylamide exposure and endometrial cancer risk.

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References

1. International Agency for Research on Cancer: *Some Industrial Chemicals*. Lyon, France: International Agency for Research on Cancer, 1994.
2. Erdreich LS and Friedman MA: Epidemiologic evidence for assessing the carcinogenicity of acrylamide. *Regul Toxicol Pharmacol* **39**, 150–157, 2004.
3. Granath F and Tornqvist M: Who knows whether acrylamide in food is hazardous to humans? *J Natl Cancer Inst* **95**, 842–843, 2003.
4. Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M: Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem* **50**, 4998–5006, 2002.
5. Hogervorst J, Duell E, Schouten L, Slimani N, van den Brandt P.: Reaction on the acrylamide and cancer review by Lipworth and colleagues. *Eur J Cancer Prev* **22**, 194–198, 2013.
6. Lipworth L, Sonderman JS, Tarone RE, and McLaughlin JK: Review of epidemiologic studies of dietary acrylamide intake and the risk of cancer. *Eur J Cancer Prev* **21**, 375–386, 2012.
7. Lipworth L, Sonderman JS, Tarone RE, and McLaughlin JK: Acrylamide: a human cancer risk? *Eur J Cancer Prev* **22**, 193–194, 2013.
8. Virk-Baker MK, Nagy TR, Barnes S, and Groopman J: Dietary acrylamide and human cancer: a systematic review of literature. *Nutr Cancer* **66**, 774–790, 2014.

9. Wilson KM, Mucci LA, Rosner BA, and Willett WC: A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancers. *Cancer Epidemiol Biomarkers Prev* **19**, 2503–2515, 2010.
10. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA.: A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev* **16**, 2304–2313, 2007.
11. Pedersen GS, Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, et al.: Dietary acrylamide intake and estrogen and progesterone receptor-defined postmenopausal breast cancer risk. *Breast Cancer Res Treat* **122**, 199–210, 2010.
12. Hogervorst JG, Baars BJ, Schouten LJ, Konings EJ, Goldbohm RA, et al.: The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. *Crit Rev Toxicol* **40**, 485–512, 2010.
13. Besaratinia A and Pfeifer GP: A review of mechanisms of acrylamide carcinogenicity. *Carcinogenesis* **28**, 519–528, 2007.
14. Pelucchi C, La Vecchia C, Bosetti C, Boyle P, Boffetta P: Exposure to acrylamide and human cancer—a review and meta-analysis of epidemiologic studies. *Ann Oncol* **22**, 1487–1499, 2011.
15. Larsson SC, Hakansson N, Akesson A, and Wolk A: Long-term dietary acrylamide intake and risk of endometrial cancer in a prospective cohort of Swedish women. *Int J Cancer* **124**, 1196–1199, 2009.
16. Obon-Santacana M, Kaaks R, Slimani N, Lujan-Barroso L, Freisling H, et al.: Dietary intake of acrylamide and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort. *Br J Cancer* **111**, 987–997, 2014.
17. Pelucchi C, Bosetti C, Galeone C, and La Vecchia C: Dietary acrylamide and cancer risk: an updated meta-analysis. *Int J Cancer* **136**, 2912–2922, 2015.
18. Pelucchi C, Galeone C, Levi F, Negri E, Franceschi S, et al.: Dietary acrylamide and human cancer. *Int J Cancer* **118**, 467–471, 2006.
19. Lucenteforte E, Talamini R, Montella M, Dal Maso L, Tavani A, et al.: Macronutrients, fatty acids and cholesterol intake and endometrial cancer. *Ann Oncol* **19**, 168–172, 2008.
20. Gnagnarella P, Parpinel M, Salvini S, Franceschi S, Palli D et al.: The update of the Italian Food Composition Database. *J Food Compos Anal* **17**, 509–522, 2004.
21. Salvini S, Parpinel M, Gnagnarella P, Maisonneuve P, Turriani A: *Banca dati di composizione degli alimenti per studi epidemiologici in Italia*. Milan, Italy: Istituto Europeo di Oncologia, 1998.
22. Decarli A, Franceschi S, Ferraroni M, Gnagnarella P, Parinell MT, et al.: Validation of a food-frequency questionnaire to assess dietary intakes in cancer studies in Italy. Results for specific nutrients. *Ann Epidemiol* **6**, 110–118, 1996.
23. Franceschi S, Negri E, Salvini S, Decarli A, Ferraroni M et al.: Reproducibility of an Italian food frequency questionnaire for cancer studies: results for specific food items. *Eur J Cancer*, **29A**, 2298–2305, 1993.
24. Agence Francaise de Sécurité Sanitaire des Aliments. Acrylamide: *point d'information n° 3*. Maisons-Alfort, France: 6 2005. Retrieved from <http://www.anses.fr/sites/default/files/documents/RCCP2002sa0300b.pdf>
25. Joint FAO/WHO Experts Committee on Food Additives: *Summary and conclusions of the sixty-fourth meeting of the joint FAO/WHO Expert Committee on Food Additives (JECFA)*. Geneva, Switzerland: FAO/WHO, 2005. Retrieved from <http://www.who.int/foodsafety/chem/chemicals/acrylamide/en/>
26. Sagratini G: HPLC–MS validation of QualisaFoo® biosensor kit for cost-effective control of acrylamide levels in Italian coffee. *Food Control* **18**, 1267–1271, 2007.
27. Tateo F, Bononi M, and Andreoli G: Acrylamide levels in cooked rice, tomato sauces and some fast food on the Italian market. *J Food Compos Anal* **20**, 232–235, 2007.
28. Breslow NE and Day NE: *Statistical Methods in Cancer Research. Vol 1: The Analysis of Case-Control Studies*. Geneva, Switzerland: IARC, 1980.
29. Pelucchi C, Galeone C, Dal Maso L, Talamini R, Montella M, et al.: Dietary acrylamide and renal cell cancer. *Int J Cancer* **120**, 1376–1377, 2007.
30. Pelucchi C, Galeone C, Talamini R, Negri E, Polesel J et al.: Dietary acrylamide and pancreatic cancer risk in an Italian case-control study. *Ann Oncol* **22**, 1910–1915, 2011.
31. Greenland S: Quantitative methods in the review of epidemiologic literature. *Epidemiol Rev* **9**, 1–30, 1987.
32. Hagmar L, Wirfalt E, Paulsson B, and Tornqvist M: Differences in hemoglobin adduct levels of acrylamide in the general population with respect to dietary intake, smoking habits and gender. *Mutat Res* **580**, 157–165, 2005.
33. Cook LS, Weiss NS, Doherty JA, and Chen C: Endometrial cancer. In: *Cancer Epidemiology and Prevention*, 3rd ed., Schottenfeld D and Fraumeni JF (eds.). New York: Oxford University Press, 2006, pp. 1027–1043.
34. D'Avanzo B, La Vecchia C, Katsouyanni K, Negri E, Trichopoulos D: An assessment, and reproducibility of food frequency data provided by hospital controls. *Eur J Cancer Prev* **6**, 288–293, 1997.
35. Konings EJ, Hogervorst JG, van Rooij L, Schouten LJ, Sizoo EA et al.: Validation of a database on acrylamide for use in epidemiological studies. *Eur J Clin Nutr* **64**, 534–540, 2010.
36. Freisling H, Moskal A, Ferrari P, Nicolas G, Knaze V, et al.: Dietary acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition differs greatly according to geographical region. *Eur J Nutr* **52**, 1369–1380, 2013.

ORIGINAL ARTICLE

Dietary acrylamide and the risk of pancreatic cancer in the International Pancreatic Cancer Case–Control Consortium (PanC4)

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Background: Occupational exposure to acrylamide was associated with excess mortality from pancreatic cancer, though in the absence of dose–risk relationship. Few epidemiological studies have examined the association between acrylamide from diet and pancreatic cancer risk.

Patients and methods: We considered this issue in a combined set of 1975 cases of pancreatic cancer and 4239 controls enrolled in six studies of the Pancreatic Cancer Case–Control Consortium (PanC4). We calculated pooled odds ratios (ORs) and their 95% confidence intervals (CI) by estimating study-specific ORs through multivariate unconditional logistic regression models and pooling the obtained estimates using random-effects models.

Results: Compared with the lowest level of estimated dietary acrylamide intake, the pooled ORs were 0.97 (95% CI, 0.79–1.19) for the second, 0.91 (95% CI, 0.71–1.16) for the third, and 0.92 (95% CI, 0.66–1.28) for the fourth (highest) quartile of intake. For an increase of 10 µg/day of acrylamide intake, the pooled OR was 0.96 (95% CI, 0.87–1.06), with heterogeneity between estimates ($I^2 = 67\%$). Results were similar across various subgroups, and were confirmed when using a one-stage modelling approach.

Conclusions: This PanC4 pooled-analysis found no association between dietary acrylamide and pancreatic cancer.

Key words: acrylamide, case–control studies, pancreatic neoplasms, pooled-analysis, risk factors

Introduction

The association between dietary acrylamide intake and risk of several common cancers has been widely debated [1–5], following the findings of a Swedish study reporting the formation of acrylamide in foods, particularly starchy foods such as potatoes and breads, cooked at high temperatures [6].

Epidemiological data on the association between dietary acrylamide and pancreatic cancer are scant. It is of particular interest to investigate this association because two studies of acrylamide exposure in the occupational setting [7,8] have shown moderately increased mortality from pancreatic cancer, in the absence of a dose–risk relationship. When the results of those studies were pooled, the summary standardized

Table 1. Information on daily acrylamide intake in the studies considered in the analyses. International Pancreatic Cancer Case–Control Consortium (PanC4)

Study centre	Mean intake±SD, cases (µg/day)	Mean intake±SD, controls (µg/day)	25th percentile (µg/day) ^a	Median intake (µg/day) ^a	75th percentile (µg/day) ^a	Mean intake±SD per kg of BW, cases (µg/day/kg BW)	Mean intake±SD per kg of BW, controls (µg/day/kg BW)
MD Anderson, USA	20.5±12.5	22.2±13.7	12.5	19.0	28.0	0.25±0.16	0.27±0.17
Italy	33.6±15.9	31.2±17.2	19.1	28.2	39.6	0.47±0.24	0.43±0.24
UCSF, USA	22.7±13.7	20.7±14.5	11.9	17.6	26.0	0.31±0.18	0.29±0.20
LSU, USA	20.3±14.8	16.8±11.2	8.3	14.7	22.2	0.25±0.18	0.22±0.16
UMN, USA	25.3±14.4	26.0±14.6	16.5	23.1	31.9	NA ^b	NA ^b
QIMR, Australia	22.8±16.6	20.0±11.1	12.5	17.8	25.5	0.29±0.22	0.26±0.16

^aAmong controls.

^bInformation on body weight was not available in the University of Minnesota study.

BW, body weight; LSU, Louisiana State University; NA, not available; QIMR, QIMR Berghofer Medical Research Institute; SD, standard deviation; UCSF, University of California, San Francisco; UMN, University of Minnesota.

mortality ratios (SMR) of pancreatic cancer were 1.54 (95% confidence interval, CI, 0.95–2.36) for any occupational exposure to acrylamide and 1.67 (95% CI, 0.83–2.99) for high exposure [4]. In contrast, a recent systematic review and meta-analysis of epidemiological data considering exposure to dietary acrylamide reported relative risks (RR) of pancreatic cancer of 0.93 (95% CI, 0.76–1.12) for high versus low acrylamide intake and of 0.99 (95% CI, 0.95–1.03) for an increase in intake equal to 10 µg/day. The estimates were based on four studies (three cohort and one case–control study), with a total of 1732 cases [5].

With the aim to provide additional evidence on this topic, given the scant amount of available epidemiological data, we examined the role of acrylamide intake on a combined set of 1975 cases of pancreatic cancer (i.e. more than those included in the meta-analysis) and over 4000 controls enrolled in six studies from the Pancreatic Cancer Case–Control Consortium (PanC4) [9].

Materials and methods

PanC4 is an International consortium of scientists set up to investigate the aetiology of pancreatic cancer, through pooled analyses of shared data (<http://www.panc4.org>; date last accessed: 12 December 2016). This investigation is based on six case–control studies [10–15] with comprehensive food frequency questionnaires, including information on the major food items contributing acrylamide intake (e.g. potato products cooked at high temperatures, breads, coffee) as well as calculation of total energy intake. They included a total of 1975 cases and 4239 controls. The main characteristics of the studies are described in [supplementary Table S1](#), available at *Annals of Oncology* online. A number of participants in three case–control studies included [11, 12, 14] lacked >10% of the required information to estimate acrylamide intake (i.e. they had not completed the food frequency questionnaires), and were therefore excluded from the analyses. Another PanC4 participating study from Shanghai, China [16], could not be included in the investigation since data on content of dietary acrylamide in Chinese foods was not available at the time of data analysis. Cases and controls were interviewed in-person in all studies except the Queensland (Australia) study, where interviews were conducted either over the telephone or face-to-face, with a self-administered food frequency questionnaire.

For the present analyses, the original datasets were restructured either by the original study investigators or by our central coordinators using a uniform format for data harmonization. From each study, individual

data on socio-demographic characteristics, anthropometric measures, tobacco smoking and history of diabetes were collected, whenever available.

The list of foods containing dietary acrylamide was derived from international databases [17–19], and included coffee, breads, potato products, various breakfast cereals, biscuits and cookies, gingerbread and spiced cakes, chocolate products (such as brownies and candy bars), several types of snacks and pastries, fried fish, fried chicken, pizza, tacos, fried rice and beer. Each participating study was asked to provide information on individual consumption of all these food items of cases and controls, whenever available in food frequency questionnaires, together with their cooking method whenever relevant (particularly for potato products). The total number of food items containing acrylamide in each study ranged from 14 (in the Louisiana State University study) to 33 (in the University of Minnesota study). Subjects who had a maximum of 10% of missing information among the food items contributing to total acrylamide were maintained in the analyses, by assigning to the missing food item the study-specific median frequency of consumption of that item. Information on total energy intake was also collected, for adjustment purposes. Data on the average acrylamide content of foods were derived from area-specific resources. Thus, for studies from USA, we applied measures of acrylamide levels made available from the United States Food and Drug Administration (Total Diet Study 2003–2006 [18]); for the Italian study, we used data from the European Food Safety Agency [17] and the Agence Française de Sécurité Sanitaire des Aliments (AFSSA) [20], integrated with estimates on specific Italian dietary items [21, 22]; and for the Australian study, we used data from two complementary reports from the Government of South Australia (on non-carbohydrate based foods [23]) and from Croft et al. (on carbohydrate based foods [24]).

Statistical analysis

A two-stage modelling approach was used to estimate the association between dietary acrylamide intake and risk of pancreatic cancer. In the first stage, we considered the association between acrylamide intake and risk of pancreatic cancer separately for each study, by estimating the odds ratios (ORs) and their 95% CI through multivariate unconditional logistic regression models [25], including *a priori* defined terms, when available, for age, sex, race/ethnicity, education, smoking habits, diabetes, body mass index, and total energy intake. The Italian study was further adjusted for two study-specific covariates, i.e. period of interview and study centre. We modelled exposure using both study-specific quartiles and a continuous measure (i.e. 10 µg/day) of acrylamide intake. In the second stage, summary (pooled) effect estimates were computed using a random-effects model [26].

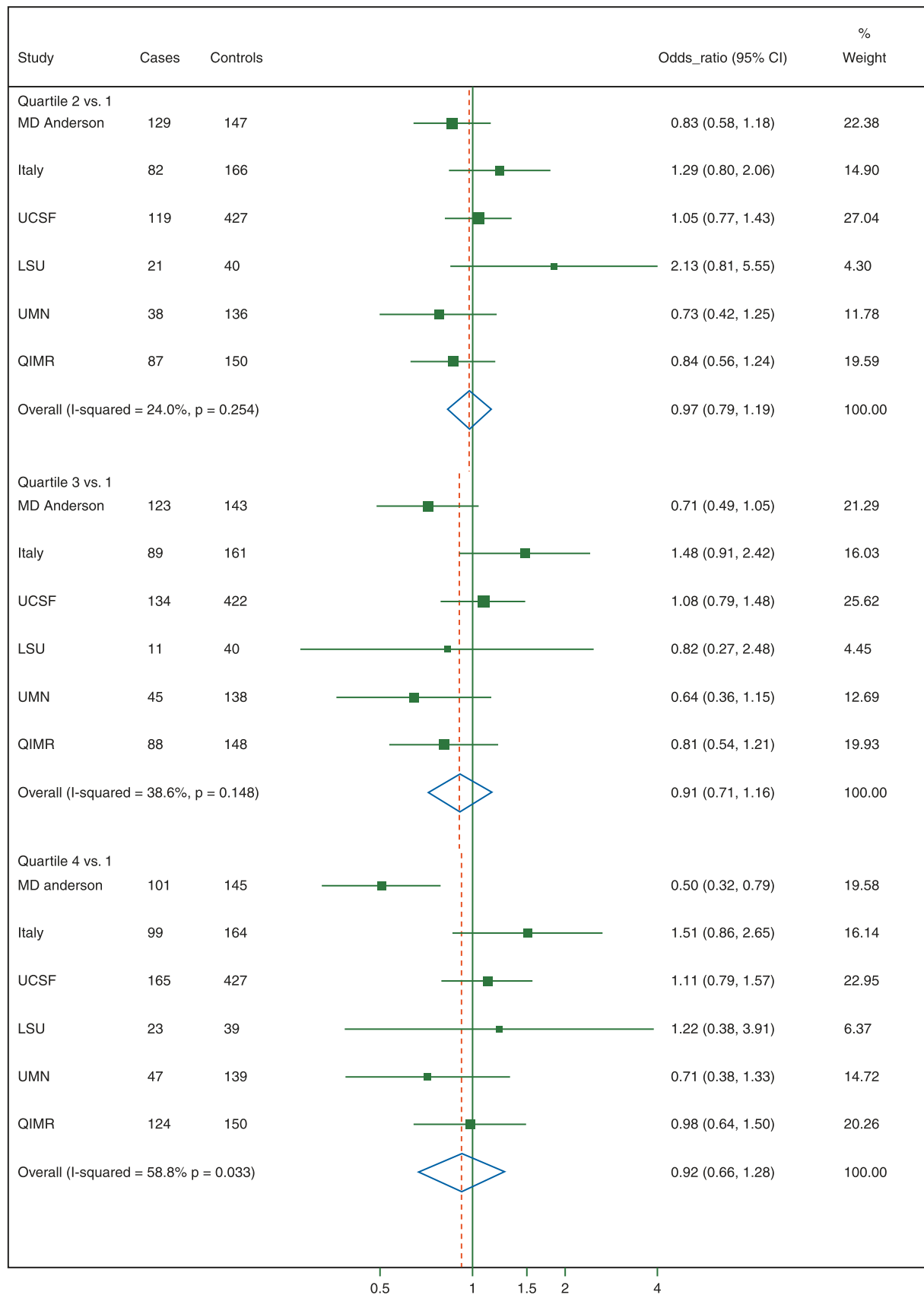


Figure 1. Study-specific and pooled odds ratios (OR), and corresponding 95% confidence intervals (CI), of pancreatic cancer according to quartiles of acrylamide intake, with the lowest intake being the reference. International Pancreatic Cancer Case–Control Consortium (PanC4).

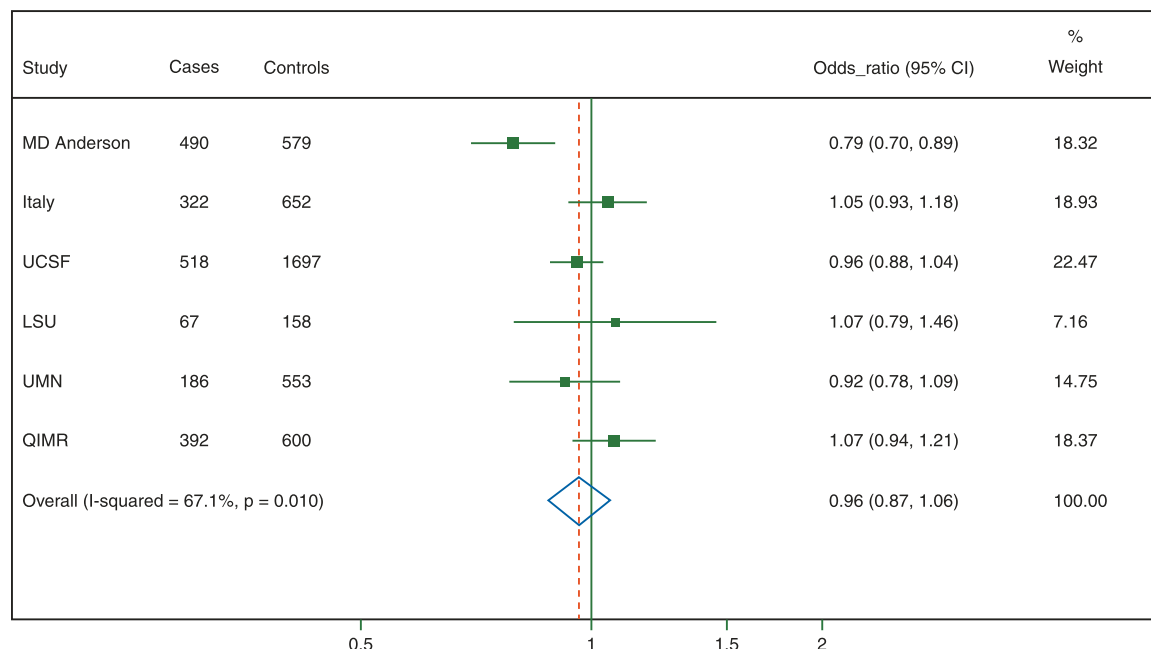


Figure 2. Study-specific and pooled odds ratios (OR), and corresponding 95% confidence intervals (CI), of pancreatic cancer according to an increase of 10 µg/day in acrylamide intake. International Pancreatic Cancer Case–Control Consortium (PanC4).

Table 2. Pooled odds ratios (OR), and corresponding 95% confidence intervals (CI), of pancreatic cancer for high versus low acrylamide intake, overall and in strata of selected covariates. International Pancreatic Cancer Case–Control Consortium (PanC4)

Covariate	Low intake (1st quartile)		High intake (4th quartile)		OR (95% CI) ^a	P value ^b
	Cases, n (%)	Controls, n (%)	Cases, n (%)	Controls, n (%)		
Overall	450 (22.8)	1057 (24.9)	559 (28.3)	1064 (25.1)	0.92 (0.66–1.28)	–
Sex						
Male	207 (18.0)	471 (20.2)	375 (32.6)	695 (29.8)	0.78 (0.44–1.36)	0.32
Female	243 (29.4)	586 (30.7)	184 (22.3)	369 (19.3)	1.16 (0.67–2.01)	
Age						
<65	181 (19.0)	469 (23.1)	299 (31.3)	549 (27.1)	0.88 (0.51–1.51)	0.83
≥65	269 (26.3)	588 (26.6)	260 (25.5)	515 (23.3)	0.94 (0.72–1.23)	
Smoking habit						
Never smokers	204 (27.7)	528 (27.8)	179 (24.3)	416 (21.9)	1.08 (0.79–1.47)	0.29
Former cigarette smokers	167 (22.4)	414 (24.0)	220 (29.4)	459 (26.6)	0.79 (0.50–1.23)	
Current cigarette smokers	72 (16.2)	95 (18.8)	144 (32.5)	164 (32.5)	0.56 (0.22–1.46)	
Body mass index (BMI) ^c						
<30 kg/m ²	315 (22.3)	768 (24.8)	402 (28.4)	779 (25.2)	0.93 (0.60–1.43)	0.94
≥30 kg/m ²	75 (20.9)	142 (24.7)	107 (29.8)	143 (24.9)	0.90 (0.46–1.78)	
Diabetes						
No	347 (22.2)	957 (25.0)	442 (28.3)	953 (24.9)	0.92 (0.65–1.30)	0.69
Yes ^d	96 (24.5)	100 (24.4)	111 (28.3)	111 (27.1)	0.79 (0.41–1.51)	
Study area						
USA (4 studies)	305 (24.2)	744 (24.9)	336 (26.6)	750 (25.1)	0.80 (0.50–1.26)	0.23
Other (2 studies)	145 (20.3)	313 (25.0)	223 (31.2)	314 (25.1)	1.17 (0.77–1.77)	

^aORs from multivariate logistic regression models adjusted for age, sex, race/ethnicity, education, smoking habits, diabetes, body mass index, and total energy intake.

^bP value for heterogeneity between strata.

^cInformation was not available in the University of Minnesota study, thus, the OR estimates on acrylamide intake in strata of BMI are based on five studies.

^dOR estimate is based on five studies, since for the LSU study, there were too few diabetic patients for the model to converge.

Heterogeneity between studies was examined using the χ^2 statistic [27] and quantified through the I^2 [28].

Subgroup analyses were conducted to examine whether the effect of high versus low acrylamide intake was heterogeneous across strata (through the χ^2 statistic [27]) of sex, age, smoking habit, obesity, diabetes and geographic area of the study.

In addition to the two-stage analysis, we performed an aggregate analysis by pooling data from all six studies into a single large dataset (one-stage analysis). The association between acrylamide intake and the risk of pancreatic cancer was then assessed through multivariate unconditional logistic regression models [25], adjusted for study and the same covariates reported above for the two-stage modelling approach. This analysis was conducted by including in the model the study-specific quartiles of acrylamide intake or, in an additional sensitivity analysis, quartiles of acrylamide intake based on the distribution of all 4239 controls. We also conducted a sensitivity analysis in which each study was excluded one at a time to ensure that the magnitude of the overall estimates was not dependent on any specific study, and another one by excluding all subjects that had any missing information in food items contributing to total acrylamide intake (i.e. about 10% of cases and 8% of controls).

Results

Supplementary Table S2, available at *Annals of Oncology* online, presents the distribution of pancreatic cancer cases and controls according to sex, age and other selected covariates.

Table 1 gives information on estimates of dietary acrylamide intake in the six studies. The mean daily intake of acrylamide in cases ranged between 20.3 μg in the Louisiana State University study and 33.6 μg in the Italian study, and in controls between 16.8 and 31.2 μg (in the same studies). The median acrylamide intake of controls varied from a lowest value of 14.7 $\mu\text{g/day}$ in the Louisiana State University study to a highest of 28.2 $\mu\text{g/day}$ in the Italian study. The main food categories contributing to total acrylamide intake in each study, and the corresponding contribution proportions, are reported in supplementary Table S3, available at *Annals of Oncology* online.

Figure 1 shows a forest plot, displaying the ORs and 95% CIs for each study and the pooled estimate, according to study-specific quartiles of acrylamide intake. Compared to the lowest study-specific quartile of dietary acrylamide intake, the pooled ORs were 0.97 (95% CI, 0.79–1.19) for the second, 0.91 (95% CI, 0.71–1.16) for the third and 0.92 (95% CI, 0.66–1.28) for the fourth (highest) quartile of intake. Heterogeneity of the risk estimates between participating studies was low for the second ($I^2=24\%$), low to moderate for the third ($I^2=39\%$) and moderate to high for the fourth quartile of intake ($I^2=59\%$) [28]. Four sensitivity analyses were conducted. In the first one, we pooled all studies into a single dataset and conducted an aggregate (one-stage) analysis. This gave an OR of 0.96 (95% CI, 0.80–1.16) for the highest versus lowest study-specific quartile of intake. The second one used the pooled dataset and calculated quartiles of intake based on the distribution in all controls, rather than being study-specific. This analysis found an OR of 0.98 (95% CI, 0.81–1.19) for the highest versus the lowest quartile. In the third one, we excluded one study at a time, and found ORs ranging from 0.84 (95% CI, 0.60–1.17, when excluding the Italian study) to 1.07 (95% CI, 0.86–1.33, when excluding the MD Anderson study) for the highest versus lowest quartile of intake. In the fourth one, we excluded all subjects that had any missing information in food items contributing to total

acrylamide: the pooled OR for high versus low acrylamide intake, based on 1766 cases and 3885 controls, was 0.96 (95% CI, 0.74–1.26).

Figure 2 shows the ORs and the corresponding 95% CIs for each study and the pooled estimate for an increase of 10 $\mu\text{g/day}$ of dietary acrylamide intake. The latter was 0.96 (95% CI, 0.87–1.06), with high heterogeneity between estimates ($I^2=67\%$). When studies were pooled into a single dataset (one-stage analysis), the OR for an increase of 10 $\mu\text{g/day}$ of dietary acrylamide intake was 0.98 (95% CI, 0.93–1.02).

Table 2 presents the results of analyses of high compared to low dietary acrylamide intake, stratified by sex, age, smoking habit, BMI, diabetes and study area. None of the summary estimates in any subgroup examined was significantly different from unity. No significant differences were observed between subgroups (all P values were >0.05).

Discussion

This large individual-level pooled-analysis from the PanC4 study reported no evidence of increased pancreatic cancer risk associated with estimated intake of acrylamide. Pooled risk estimates for increasing acrylamide intake were below unity, and none of the studies included showed significant positive associations between dietary acrylamide and pancreatic cancer. These findings were not substantively changed by a number of sensitivity analyses, and were consistent in strata of smoking habit and other individual-level and study-level covariates.

Only three cohort studies [29–31] and one case–control study (included in this investigation, too [32]) have previously reported results on acrylamide from diet in relation to the risk of pancreatic cancer. None showed increases in risk in participants with high compared to low acrylamide intake. The largest analysis, from the European Prospective Investigation into Cancer and Nutrition (EPIC) study, was based on 865 cases and reported ORs of 0.77 (95% CI, 0.58–1.04) for high versus low acrylamide and 0.95 (95% CI, 0.89–1.01) for each 10 $\mu\text{g/day}$ increase in intake [30]. Overall, our results are consistent with those from a meta-analysis of the four previous studies [5], thus confirming in a larger dataset—and with the advantages of an individual data approach—the lack of an association between acrylamide intake and pancreatic cancer risk. With further reference to potential associations in specific population subgroups, an inverse relation emerged among obese subjects in the EPIC analysis [30]. This was not, however, confirmed in our dataset.

We computed the estimates of acrylamide intake using food frequency questionnaires combined with databases of mean content of acrylamide in foods. Still, the amount of acrylamide varies widely within different samples of the same food item, due to variations in cooking procedures (e.g. duration of cooking and temperature), food characteristics (e.g. potato variety) and storage, or product brand (e.g. for breakfast cereals and chips). Further, we estimated intakes in populations from different continents, enrolled during different time periods, and acrylamide food contents vary across geographic areas (e.g. higher values for white bread in Europe than in USA) and over the years. We tried to overcome these problems by using area-specific databases of acrylamide content in foods. As a consequence, a Chinese study of the PanC4 [16] was not included in this investigation, since area-specific information on acrylamide content in food was not available at the time of

analysis. With reference to variations in acrylamide food contents over the years, manufacturers' measurements reported a substantial downward trend since 2002, more for potato crisps/chips than for other foods [33]. None of the acrylamide-rich foods, and notably coffee, has in any case been associated with pancreatic cancer risk [34]. Early symptoms of disease might also have led to diet modifications in pancreatic cancer cases. Epidemiological studies using biomarkers of acrylamide exposure would be needed to overcome most of these limitations. Strengths of this study are its large size and the consortium, individual-level, data approach, with consequent availability of detailed and harmonized information for relevant covariates. Also, we performed various sensitivity analyses to assess the robustness of results, and no meaningful differences emerged.

Acrylamide may play a role in the aetiology of cancer through its oxidation to glycidamide, a chemically reactive genotoxic metabolite [35], and—for selected body sites, such as endometrial and ovarian cancer—by affecting hormonal balances in humans [36]. To date, however, epidemiological studies found no evidence of any relationship for cancers of the digestive organs [5]. Findings from this PanC4 pooled-analysis further support a lack of association between dietary acrylamide and pancreatic cancer.

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Disclosure

The authors have declared no conflicts of interest.

References

- Hogervorst J, Duell E, Schouten L et al. Reaction on the acrylamide and cancer review by Lipworth and colleagues. *Eur J Cancer Prev* 2013; 22: 194–198.
- Lipworth L, Sonderman JS, Tarone RE, McLaughlin JK. Review of epidemiologic studies of dietary acrylamide intake and the risk of cancer. *Eur J Cancer Prev* 2012; 21: 375–386.
- Lipworth L, Sonderman JS, Tarone RE, McLaughlin JK. Acrylamide: a human cancer risk?. *Eur J Cancer Prev* 2013; 22: 193–194.
- Pelucchi C, La Vecchia C, Bosetti C et al. Exposure to acrylamide and human cancer—a review and meta-analysis of epidemiologic studies. *Ann Oncol* 2011; 22: 1487–1499.
- Pelucchi C, Bosetti C, Galeone C, La Vecchia C. Dietary acrylamide and cancer risk: an updated meta-analysis. *Int J Cancer* 2015; 136: 2912–2922.
- Tareke E, Rydberg P, Karlsson P et al. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem* 2002; 50: 4998–5006.
- Marsh GM, Youk AO, Buchanich JM et al. Mortality patterns among workers exposed to acrylamide: updated follow up. *J Occup Environ Med* 2007; 49: 82–95.
- Swaen GM, Haidar S, Burns CJ et al. Mortality study update of acrylamide workers. *Occup Environ Med* 2007; 64: 396–401.
- Bertuccio P, La Vecchia C, Silverman DT et al. Cigar and pipe smoking, smokeless tobacco use and pancreatic cancer: an analysis from the International Pancreatic Cancer Case-Control Consortium (PanC4). *Ann Oncol* 2011; 22: 1420–1426.
- Talamini R, Polesel J, Gallus S et al. Tobacco smoking, alcohol consumption and pancreatic cancer risk: a case-control study in Italy. *Eur J Cancer* 2010; 46: 370–376.
- Luckett BG, Su LJ, Rood JC, Fonthan ET. Cadmium exposure and pancreatic cancer in South Louisiana. *J Environ Public Health* 2012; 2012: 180186.
- Hassan MM, Bondy ML, Wolff RA et al. Risk factors for pancreatic cancer: case-control study. *Am J Gastroenterol* 2007; 102: 2696–2707.
- Chan JM, Wang F, Holly EA. Sweets, sweetened beverages, and risk of pancreatic cancer in a large population-based case-control study. *Cancer Causes Control* 2009; 20: 835–846.
- Tran B, Whiteman DC, Webb PM et al. Association between ultraviolet radiation, skin sun sensitivity and risk of pancreatic cancer. *Cancer Epidemiol* 2013; 37: 886–892.
- Zhang J, Dhakal IB, Gross MD et al. Physical activity, diet, and pancreatic cancer: a population-based, case-control study in Minnesota. *Nutr Cancer* 2009; 61: 457–465.
- Ji BT, Chow WH, Dai Q et al. Cigarette smoking and alcohol consumption and the risk of pancreatic cancer: a case-control study in Shanghai, China. *Cancer Causes Control* 1995; 6: 369–376.
- European Food Safety Agency (EFSA). Results on acrylamide levels in food from monitoring years 2007–2009 and exposure assessment. *EFSA J* 2011; 9: 2133.
- Food and Drug Administration (FDA). Survey data on acrylamide in food: total diet study results. 2006. <http://www.fda.gov/Food/FoodborneIllnessContaminants/ChemicalContaminants/ucm053566.htm> (12 December 2016, date last accessed).
- FAO/WHO. Summary and conclusions of the sixty-fourth meeting of the joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva: FAO/WHO 2005.
- Agence Française de Sécurité Sanitaire des Aliments (AFSSA). Acrylamide: point d'information n° 3. Maisons-Alfort: AFSSA 2005. <http://www.anses.fr/sites/default/files/documents/RCCP2002sa0300b.pdf> (12 December 2016, date last accessed).
- Tateo F, Bononi M, Andreoli G. Acrylamide levels in cooked rice, tomato sauces and some fast food on the Italian market. *J Food Compos Anal* 2007; 20: 232–235.
- Sagratini G. HPLC–MS validation of QualisaFoo® biosensor kit for cost-effective control of acrylamide levels in Italian coffee. *Food Control* 2007; 18: 1267–1271.
- Government of South Australia (GSA). A survey of acrylamide in non-carbohydrate based foods. Food Policy and Programs Branch, Public Health; Department of Health; Government of South Australia 2006.
- Croft M, Tong P, Fuentes D, Hambridge T. Australian survey of acrylamide in carbohydrate-based foods. *Food Addit Contam* 2004; 21: 721–736.
- Breslow NE, Day NE. Statistical methods in cancer research. Vol 1: The analysis of case-control studies. Geneva: IARC Science Publication 1980.
- DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; 7: 177–188.
- Greenland S. Quantitative methods in the review of epidemiologic literature. *Epidemiol Rev* 1987; 9: 1–30.
- Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; 327: 557–560.
- Hogervorst JG, Schouten LJ, Konings EJ et al. Dietary acrylamide intake is not associated with gastrointestinal cancer risk. *J Nutr* 2008; 138: 2229–2236.

30. Obon-Santacana M, Slimani N, Lujan-Barroso L et al. Dietary intake of acrylamide and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. *Ann Oncol* 2013; 24: 2645–2651.
31. Hirvonen T, Kontto J, Jestoi M et al. Dietary acrylamide intake and the risk of cancer among Finnish male smokers. *Cancer Causes Control* 2010; 21: 2223–2229.
32. Pelucchi C, Galeone C, Talamini R et al. Dietary acrylamide and pancreatic cancer risk in an Italian case–control study. *Ann Oncol* 2011; 22: 1910–1915.
33. EFSA. Scientific Opinion on acrylamide in food. EFSA Panel on Contaminants in the Food Chain (CONTAM). *EFSA J* 2015; 13: 4104.
34. World Cancer Research Fund (WCRF) and American Institute for Cancer Research (AICR). Continuous Update Project Report. Food, Nutrition, Physical Activity, and the Prevention of Pancreatic Cancer, 2012. <http://wcrf.org/int/research-we-fund/continuous-update-project-findings-reports/pancreatic-cancer> (12 December 2016, date last accessed).
35. International Agency for Research on Cancer (IARC). Some industrial chemicals. IARC Monographs on the evaluation of carcinogenic risks to humans, Vol. 60. Lyon: IARC 1994.
36. Hogervorst JG, Baars BJ, Schouten LJ et al. The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. *Crit Rev Toxicol* 2010; 40: 485–512.

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The Role of Genetic Variants in the Association between Dietary Acrylamide and Advanced Prostate Cancer in the Netherlands Cohort Study on Diet and Cancer

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ABSTRACT

To investigate the association between dietary acrylamide and advanced prostate cancer, we examined acrylamide-gene interactions for advanced prostate cancer risk by using data from the Netherlands Cohort Study.

Participants ($n = 58,279$ men) completed a baseline food frequency questionnaire (FFQ), from which daily acrylamide intake was calculated. At baseline, 2,411 men were randomly selected from the full cohort for case-cohort analysis. Fifty eight selected single nucleotide polymorphisms (SNPs) and two gene deletions in genes in acrylamide metabolism, DNA repair, sex steroid systems, and oxidative stress were analyzed. After 20.3 years of follow-up, 1,608 male subcohort members and 948 advanced prostate cancer cases were available for Cox analysis.

Three SNPs showed a main association with advanced prostate cancer risk after multiple testing correction: catalase (*CAT*) rs511895, prostaglandin-endoperoxide synthase 2 (*PTGS2*) rs5275, and xeroderma pigmentosum group C (*XPC*) rs2228001. With respect to acrylamide-gene interactions, only rs1800566 in *NAD(P)H quinone dehydrogenase 1* (*NQO1*) and rs2301241 in *thioredoxin* (*TXN*) showed a nominally statistically significant multiplicative interaction with acrylamide intake for advanced prostate cancer risk. After multiple testing corrections, none were statistically significant.

In conclusion, no clear evidence was found for interaction between acrylamide intake and selected genetic variants for advanced prostate cancer risk.

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Introduction

Prostate cancer is a hormone-related cancer that is responsive to androgen deprivation (hormonal) therapy (1). In the western world, prostate cancer has one of the highest incidence rates of all cancers in men, with approximately 759,000 new cases in 2012 (2). Age, family history of prostate cancer, and black race are accepted risk factors, but other risk factors have not been convincingly established (3). Incidence rates (both overall and age-specific) vary widely between countries, which can partly be explained by the increase of prostate-specific-antigen (PSA) testing in developed countries (4). Environmental factors may also contribute to these differences. For example, migrant studies have shown that prostate cancer incidence rates increased among men who migrated to a region with higher prostate

cancer incidence (5). Of course, increased access of migrants to PSA testing may contribute to this rise in incidence, but dietary factors are also believed to influence the incidence of prostate cancer (6). However, to date, there is still little known about a possible association between diet and prostate cancer (3).

Since its discovery in food in 2002, dietary acrylamide has been the subject of numerous epidemiologic studies on cancer. Acrylamide arises as a by-product of the Maillard reaction between the amino acid asparagine and reducing sugars (e.g., fructose, sucrose), during high-temperature cooking of foods such as cookies, potato chips, and French fries. The International Agency for Research on Cancer (IARC) classified acrylamide as a probable human carcinogen, based on evidence derived

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Supplemental data for this article can be accessed on the publisher's website.

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from rodent studies. Epidemiologic studies in humans, thus far, have reported inconsistent findings on cancer risk, with some studies showing increased risk for hormone-related cancers (endometrial and ovarian cancer) (7). In a previous study by our group (8), high intake of acrylamide was non-significantly inversely associated with advanced prostate cancer among never-smokers after 13.3 years of follow-up. The analysis was restricted to never-smokers to exclude any possible confounding effect of smoking, which is a major source of acrylamide. While another cohort study (9) also found acrylamide intake to be non-significantly inversely associated with advanced prostate cancer risk in never-smokers, the third other cohort study (10) did not show any associations with advanced prostate cancer. It thus remains unclear whether acrylamide intake influences advanced prostate cancer risk.

A number of mechanisms may explain the effect of acrylamide on cancer risk (11). The first mechanism involves glycidamide, an epoxide metabolite of acrylamide. Glycidamide forms DNA adducts and is therefore thought to be the carcinogenic compound in acrylamide-induced carcinogenesis due to its genotoxicity (12). Second, in previous analyses by our group (8, 13), findings with hormone-related cancers support the hypothesis of a hormonal mechanism of acrylamide. A third mechanism is acrylamide-induced oxidative stress. Oxidative stress occurs when reactive oxygen species (ROS), generated by pro-oxidants, outbalance the antioxidant system (14). This imbalance becomes more common with increasing age (15) and may therefore play an important role in the development and progression of age-related cancers including prostate cancer (16). However, it is unclear whether and how these mechanisms may provide a causal explanation for the association between acrylamide and prostate cancer.

Therefore, in the current study, we used data from the Netherlands Cohort Study on diet and cancer and explored whether genetic variation modifies the association between dietary acrylamide and advanced prostate cancer risk. For that matter, 60 single nucleotide polymorphisms (SNPs) and two gene deletions in genes involved in acrylamide metabolism and the hypothesized mechanisms of acrylamide-induced carcinogenesis (DNA repair, a sex hormonal effect and oxidative stress) were selected. We examined the association between selected genetic variants and advanced prostate cancer risk and investigated acrylamide-gene interactions.

Methods

Study Population and Design

The prospective Netherlands Cohort Study (NLCS) on diet and cancer included 58,279 men aged 55–69 years. At

baseline (1986), participants completed a one-time self-administered questionnaire on dietary habits, lifestyle, and other risk factors for cancer. Participants provided informed consent for study participation by completing and returning this questionnaire. About 75% of the participants provided toenail clippings for DNA-analyses. For reasons of efficiency, a case-cohort approach was used (17). To this end, a subcohort, including 2,411 men, was randomly selected from the full cohort immediately after baseline. Subcohort members were then followed up for migration and vital status, to accurately estimate the accumulated person years of the full cohort. Advanced prostate cancer cases were derived from the full cohort and identified by regular record linkage to the Netherlands Cancer Registry and the Dutch Pathology Registry (PALGA) (18). Further details on the study can be found elsewhere (19). The NLCS has been approved by the institutional review boards of the University Hospital Maastricht and TNO Nutrition and Food Research.

Cases were classified by the Netherlands Cancer Registry (NCR) according to the International Union Against Cancer tumor-node metastasis classification (TNM) staging system (20). We included prostate cancers with a pathologic or clinical TNM staging score of T3/T4, N+, or M1 at diagnosis. Prevalent cancer cases (other than skin cancer) at baseline were excluded from analysis. Furthermore, cases and subcohort members were excluded if dietary data were either incomplete or inconsistent, toenail clippings were not provided or genotyping was unsuccessful (sample call rate < 95%). With respect to acrylamide-gene interaction analysis, cases and subcohort members were additionally excluded if they had missing data on covariables. After 20.3 years of follow-up, 1,608 subcohort members and 948 incident cases of advanced prostate cancer were available for analysis. Figure 1 shows a flow diagram of the exclusion criteria applied to cases and subcohort members.

Assessment of Acrylamide Intake

The baseline questionnaire included a 150-item food frequency questionnaire (FFQ) to estimate daily food and nutrient intake, which has been tested for validity and reproducibility (21,22). As described in detail elsewhere (13), we used data on acrylamide levels in foods on the Dutch market. Daily acrylamide intake was estimated by multiplying frequency of consumption by portion size and the mean acrylamide content of each acrylamide-containing food.

A 24-hour duplicate diet study by our group indicated that subjects can be reliably ranked with respect to acrylamide intake using mean acrylamide values for individual foods (23). The Spearman's correlation between the

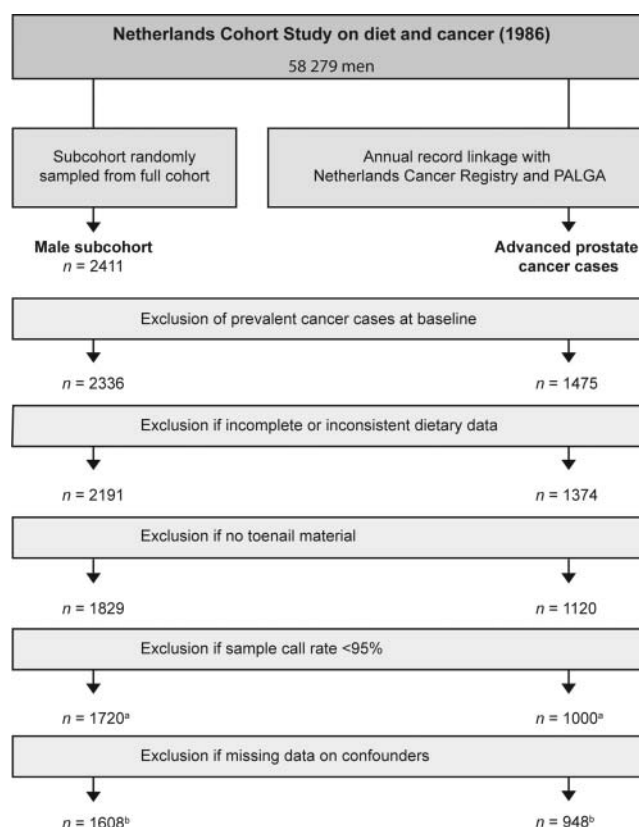


Figure 1. Flow diagram of subcohort members and advanced prostate cancer cases for 20.3 years of follow-up; Netherlands Cohort Study on diet and cancer (1986–2006). (a) Analysis on the association between selected genetic variants and advanced prostate cancer risk including 1,720 subcohort members and 1,000 advanced prostate cancer cases. (b) Analysis on the interaction between selected genetic variants and acrylamide intake on advanced prostate cancer risk including 1,608 subcohort members and 948 advanced prostate cancer cases.

calculated and (chemically) measured acrylamide intake was 0.82 ($P < 0.001$).

Gene and SNP Selection

A detailed description of the gene and SNP selection has been provided elsewhere (24). Briefly, we selected SNPs in genes involved in acrylamide metabolism and the hypothesized mechanisms of acrylamide-induced carcinogenesis: genotoxicity (selected SNPs in DNA repair genes), a sex hormonal effect and oxidative stress, and that were shown to be associated with a sex hormone-related cancer (endometrial, ovarian, breast, or prostate cancer). In addition, we selected SNPs that, to our knowledge, have not been evaluated for their association with hormone-related cancers, but were shown to be of significance in acrylamide-related polymorphism- or gene expression studies.

Only validated SNPs with a minor allele frequency of $\geq 10\%$ in Caucasians in dbSNP were selected.

The SNPs ($n = 60$) we selected were in the following genes: *aldo-keto reductase family 1, member C1* (AKR1C1), *aldo-keto reductase family 1, member C2*

(AKR1C2), *catalase* (CAT), *catechol-O-methyltransferase* (COMT), *cytochrome P450 family 1 subfamily A member 1* (CYP1A1), *cytochrome P450 family 1 subfamily A member 2* (CYP1A2), *cytochrome P450 family 1 subfamily B member 1* (CYP1B1), *cytochrome P450 family 11 subfamily A member 1* (CYP11A1), *cytochrome P450 family 17 subfamily A member 1* (CYP17A1), *cytochrome P450 family 19 subfamily A member 1* (CYP19A1), *cytochrome P450 2E1* (CYP2E1), *epoxide hydrolase 1* (EPHX1), *estrogen receptor 1* (ESR1), *estrogen receptor 2* (ESR2), *glutathione peroxidase 1* (GPX1), *glutathione S-transferase alpha 5* (GSTA5), *glutathione S-transferase P1* (GSTP1), *hydroxysteroid (17-beta) dehydrogenase 3* (HSD17B3), *3beta-hydroxysteroid dehydrogenase* (HSD3B1/B2), *MGC12965*, *mutY DNA glycosylase* (MUTYH), *nuclear factor kappa B subunit 1* (NFKB1), *nitric oxide synthase 2* (NOS2), *NAD (P)H quinone dehydrogenase 1* (NQO1), *8-oxoguanine DNA glycosylase 1* (OGG1), *progesterone receptor* (PGR), *prostaglandin-endoperoxide synthase 2* (PTGS2), *ribonucleotide reductase regulatory subunit M2* (RRM2), *sex hormone binding globulin* (SHBG), *solute carrier family 7 (cationic amino acid transporter, γ^+ system), member 11* (SLC7A11), *superoxide dismutase 1* (SOD1), *superoxide*

dismutase 2 (SOD2), steroid 5 alpha-reductase 1 (SRD5A1), sulfotransferase family 1A member 1 (SULT1A1), sulfotransferase family 1E member 1 (SULT1E1), thioredoxin (TXN), UDP glucuronosyltransferase family 1 member A6-10 (UGT1A6-10), xeroderma pigmentosum, complementation group C (XPC), and x-ray repair complementing defective repair in Chinese hamster cells 1 (XRCC1).

In addition, glutathione s-transferase mu 1 (*GSTM1*) and glutathione s-transferase theta 1 (*GSTT1*) were selected as genes involved in acrylamide metabolism (25). Since the beginning and ending of the *GSTM1* and *GSTT1* deletions are not exactly known, it was impossible to design one SNP assay (based on single base extension) for the deletion. Therefore, we chose three SNPs for *GSTM1* (rs10857795, rs200184852, and rs74837985) and four SNPs for *GSTT1* (rs2844008, rs4630, rs140309, and rs8140585) to represent the presence or absence of the gene. In case all SNPs within a gene were not called, we interpreted this as a deletion of the gene.

Finally, 67 SNPs (60 SNPs, plus 7 SNPs to represent the *GST* deletions) were genotyped using two multiplex panels. Supplementary Table S1 provides an overview of the genotyped SNPs (not including the seven SNPs representing the *GST* deletions).

DNA Isolation and Genotyping

DNA was isolated from 15 mg of toenail clippings, according to a protocol described in detail elsewhere (26). SNP genotyping was done on the MassARRAY system in conjunction with the iPLEX™ assay (27).

The reproducibility of genotyping for the analyzed SNPs (minus 7 SNPs representing the *GST* deletions) was assessed from 146 duplicate samples, which was >99% (excluding missing values). Out of 60 SNPs, two SNPs (rs3736599 and rs7741) were excluded from analyses due to insufficient genotyping success (call rate < 80%); the assay for rs3736599 failed completely (0% call rate). After correction for multiple testing, using the Benjamini–Hochberg (1995) false discovery rate (FDR) approach (28), two SNPs (rs1001179 and rs5746136) were not in Hardy–Weinberg equilibrium (FDR-adjusted P value < 0.20) (see Supplementary Table S1).

A total of 229 samples (120 cancer cases, 109 subcohort members) were excluded due to a sample call rate below 95%. With respect to the three selected SNPs to represent the *GSTM1* deletion, rs10857795 was not called in 39%, rs200184852 in 44%, and rs74837985 in 2% of the subcohort. The *GSTM1* gene is deleted in approximately 40–50% of the Caucasians. This probably indicates that the low proportion of missings for rs74837985 was due to genotyping error, possibly caused by unspecific amplification. Therefore, only rs10857795

and rs200184852 were selected to represent the *GSTM1* deletion. With respect to the four SNPs representing the *GSTT1* deletion, it was found that rs2844008 was not called in 64%, rs4630 in 15%, rs140309 in 11%, and rs8140585 in 85% of the subcohort. The *GSTT1* gene is deleted in about 20% of the Caucasians, thus rs2844008 and rs814058 were probably not correctly genotyped and therefore not statistically analyzed in isolation.

Statistical Analysis

A Cox proportional hazards model was used to calculate hazard ratios (HRs) with 95% confidence intervals (CIs). Robust standard errors were calculated to account for the additional variance introduced by sampling a subcohort from the full cohort (29). Follow-up time (time-on-study) was used as the time scale and defined as time from baseline (Sept. 1986) to either diagnosis of advanced prostate cancer, death, emigration or loss to follow-up, whichever came first. The proportional hazards (PH) assumption was assessed by using the scaled Schoenfeld residuals (30).

In models that examined the main effect of dietary acrylamide and acrylamide-gene interactions, age, family history of prostate cancer, and smoking were included as predefined covariables. Internal acrylamide exposure and smoking are strongly associated, because smoking is an important source of acrylamide. For smoking to be a confounder, it must be associated with advanced prostate cancer risk as well. The evidence for this association is mixed (31,32) and unpublished results by our group did not reveal an association between smoking and advanced prostate cancer risk. However, to minimize any residual confounding, we decided to adjust for smoking. In this perspective, we also analyzed never-smokers (main effect dietary acrylamide only) and non-smokers (never smokers combined with former smokers who had quit smoking more than 10 years before baseline). Preferably, we would have analyzed the never-smokers group for acrylamide-gene interactions (given the previously reported inverse association; ref. 8), but this was impossible due to the insufficient number of cases and subcohort members in this group. Therefore, we chose to analyze non-smokers and also adjusted for (former) smoking within that group. The smoking variables that were entered into the models were: cigarette smoking status (never/former/current), frequency of cigarettes smoked per day, and duration of smoking (years).

Based on literature, the following variables were *a priori* considered as potential confounders and only included in the model if they changed the acrylamide hazard ratio by >10%: BMI (kg/m²), non-occupational physical activity (min/day), level of education (four

categories), positive history of diabetes (yes/no), total energy intake (kcal/day), fruit (g/day), vegetables (g/day), dairy products (g/day), lycopene ($\mu\text{g/day}$), calcium (mg/day), and vitamin E ($\mu\text{g/day}$). None of the variables changed the acrylamide hazard ratio by $>10\%$ and were thus not included in the final model. Throughout the analyses, we adjusted for age, family history of prostate cancer, and smoking, except for the association between SNPs and advanced prostate cancer risk (age-adjusted only).

As a first analysis, we examined whether the inverse association between dietary acrylamide and advanced prostate cancer risk in never-smokers after 13.3 years of follow-up persisted after 20.3 years of follow-up.

The association between variants in *CAT* (rs1001179), *GPX1* (rs3448), *NQO1* (rs1800566), *OGG1* (rs1052133), *SOD1* (rs10432782), and *SOD2* (rs4880) and advanced prostate cancer risk were previously reported by our group (33,34). For that reason, the main effects of these SNPs on advanced prostate cancer risk will not be presented in the current study.

Multiplicative interaction P values for the interaction between acrylamide and genotypes (assuming a dominant genetic model) were tested using product terms between acrylamide intake (continuous) and genotype. Dose-response across genotype strata was tested by using the median acrylamide intake of each quartile as a continuous variable. In sensitivity analysis, we repeated the acrylamide-gene interaction analysis in non-smokers for the 13.3 year follow-up period. P trends (main effect SNPs only) and acrylamide-gene interaction P values were corrected for multiple testing, using the Benjamini-Hochberg (1995) FDR approach (28). The FDR threshold for these analyses was set at 0.20, which is common in candidate gene studies (33). FDR-adjusted P values were separately calculated for the total study population and for non-smokers.

All statistical analyses were performed with STATA (version 13.1, StataCorp LP, College Station, TX, USA) and reported P values were two-sided, with $P < 0.05$ considered nominally statistically significant.

Results

At baseline, cases were comparable to subcohort members regarding acrylamide intake, age, BMI, education, cigarette smoking status, and diet (Table 1). In the subcohort, former smokers with more than 10 years of cessation had quit smoking for a mean (SD) of 20.8 (7.0) years, which was comparable to that of cases. As compared to subcohort members, cases more often had a family history of prostate cancer but less often a history of diabetes. Subcohort members (and cases) that provided toenail clipping were comparable to subcohort

Table 1. Baseline characteristics of subcohort members and advanced prostate cancer cases in the Netherlands Cohort Study on diet and cancer (1986–2006)¹.

Variable	Subcohort (n = 1,608)	Cases (n = 948)
Acrylamide ($\mu\text{g/day}$)	22.4 (11.9)	22.9 (12.2)
Age (years)	61.2 (4.2)	61.7 (4.1)
BMI (kg/m^2)	24.9 (2.6)	25.0 (2.4)
Non-occupational physical activity (min/day) ¹	63 (37–103)	64 (41–103)
Level of education (%)		
Primary school	23.9	24.3
Lower vocational	20.8	18.0
High school	35.2	35.7
Higher vocational/university	20.1	22.0
Cigarette smoking status (%)		
Never smoker	13.8	14.7
Former smoker	54.2	57.2
Current smoker	32.0	28.2
Former smokers >10 years cessation		
Frequency of cigarette smoking (n/day)	16.4 (11.8)	15.5 (10.4)
Duration of cigarette smoking (years)	22.4 (8.5)	22.7 (8.3)
Time since cessation (years)	20.8 (7.0)	20.6 (7.0)
Positive history of diabetes (%)	3.1	2.2
Family history of prostate cancer (%)	2.5	3.5
Dietary intake		
Total energy intake (kcal/day)	2,155 (498)	2,166 (486)
Fruit (g/day) ²	137 (78–209)	142 (79–215)
Vegetables (g/day)	193 (84)	198 (82)
Dairy products (g/day) ²	266 (165–399)	291 (181–419)
Lycopene ($\mu\text{g/day}$) ²	751 (363–1,237)	802 (389–1,366)
Calcium (mg/day)	944 (341)	971 (339)
Vitamin E (mg/day)	14.7 (6.6)	15.2 (6.6)

¹Values are means \pm SEMs or percentages unless otherwise indicated.

²Values are medians; ranges in parentheses.

members (and cases) that did not provide toenail clippings, except for level of education and cigarette smoking status (data not shown).

Main Effect of Acrylamide Intake

After 20.3 years of follow-up no associations were found between acrylamide intake and advanced prostate cancer risk in the total study population [HR(Q5 vs. Q1) = 1.03, 95% CI: 0.82–1.29; P trend = 0.89], never-smokers [HR(Q5 vs. Q1) = 0.90, 95% CI: 0.51–1.60; P trend = 0.68] and non-smokers [HR(Q5 vs. Q1) = 0.93, 95% CI: 0.68–1.26; P trend = 0.78] (Table 2). Also, analysis with 13.3 years of follow-up did not reveal any association apart from the previously reported (8) non-statistically significant inverse dose response relationship in never-smokers (Table 2).

Main Effect of SNPs

Six SNPs showed a nominally statistically significant association with advanced prostate cancer risk after 20.3 years of follow-up (Table 3). Men with variant alleles of rs11252887 (*AKRIC2*), rs511895 (*CAT*),

Table 2. Association between dietary acrylamide intake and advanced prostate cancer risk after 13.3 and 20.3 years of follow-up; Netherlands Cohort Study on diet and cancer (1986).

Study population	Follow-up	Acrylamide, continuous		Acrylamide, quintiles of intake											
		n cases	total	10 μ g/day HR (95% CI) ¹	Q1		Q2		Q3		Q4		Q5		
					n cases	HR (95% CI) ¹	n cases	HR (95% CI) ¹	n cases	HR (95% CI) ¹	n cases	HR (95% CI) ¹	n cases	HR (95% CI) ¹	P trend
Total	20.3y	1,290		1.02 (0.96–1.08)	267	1.00 (ref)	264	1.05 (0.83–1.32)	242	0.95 (0.75–1.20)	245	0.99 (0.78–1.25)	272	1.03 (0.82–1.29)	0.89
	13.3y	813		1.01 (0.94–1.08)	169	1.00 (ref)	183	1.17 (0.90–1.51)	144	0.95 (0.72–1.25)	150	1.02 (0.78–1.34)	167	1.04 (0.80–1.36)	0.97
Never-smokers	20.3y	190		1.00 (0.85–1.17)	47	1.00 (ref)	41	0.96 (0.54–1.71)	30	0.94 (0.50–1.77)	31	0.85 (0.47–1.55)	41	0.90 (0.51–1.60)	0.68
	13.3y	121		0.90 (0.74–1.09)	33	1.00 (ref)	30	1.04 (0.55–1.98)	18	0.89 (0.43–1.84) ²	18	0.81 (0.40–1.65)	22	0.68 (0.34–1.36)	0.19
Non-smokers ³	20.3y	670		1.00 (0.92–1.08)	161	1.00 (ref)	119	0.83 (0.60–1.15)	128	1.01 (0.73–1.41) ²	114	0.86 (0.62–1.20)	148	0.93 (0.68–1.26)	0.78
	13.3y	409		0.99 (0.90–1.09)	98	1.00 (ref)	81	0.97 (0.67–1.41)	73	1.05 (0.71–1.55)	73	1.01 (0.68–1.48)	84	0.93 (0.64–1.34)	0.71

Abbreviations: CI, confidence interval; HR, hazard ratio.

¹Hazard ratios adjusted for age, family history of prostate cancer, cigarette smoking status (never/former/current), frequency of smoking (number of cigarettes per day; centered), and duration of smoking (number of years; centered).

²Possible violation of the proportional hazards assumption but no statistically significant interaction with time (P value \geq 0.05).

³Never smokers combined with former smokers who had quit smoking more than 10 years before baseline.

Table 3. SNPs showing nominally statistically significant association (P trend < 0.05) with advanced prostate cancer risk after 20.3 years of follow-up; Netherlands Cohort Study on diet and cancer (1986–2006).

Gene	SNP ¹	Genotype	Person-years	Advanced prostate cancer			
				n cases	HR (95% CI) ²	P trend	FDR-adjusted P value ³
<i>AKR1C2</i>	rs11252887	CC	13 828	464	1.00 (ref)	0.02	0.26
		CT	11 065	422	1.14 (0.96–1.35)		
		TT	2191	101	1.38 (1.03–1.85)		
		Per minor allele	27 084	987	1.16 (1.03–1.32)		
<i>CAT</i>	rs511895	GG	9882	308	1.00 (ref)	0.01	0.15
		AG	12 786	495	1.25 (1.04–1.50)		
		AA	4691	197	1.36 (1.07–1.71)		
		Per minor allele	27 359	1000	1.17 (1.05–1.31)		
<i>HSD3B1/B2</i>	rs10923823	TT	9270	314	1.00 (ref)	0.03	0.29
		CT	13 032	467	1.06 (0.88–1.27)		
		CC	5021	218	1.31 (1.04–1.64)		
		Per minor allele	27 323	999	1.14 (1.01–1.27)		
<i>HSD3B1/B2</i>	rs7546652	TT	9279	313	1.00 (ref)	0.03	0.29
		CT	13 080	469	1.06 (0.88–1.28)		
		CC	5001	218	1.32 (1.05–1.65)		
		Per minor allele	27 359	1,000	1.14 (1.02–1.28)		
<i>PTGS2</i>	rs5275	TT	12 500	493	1.00 (ref)	0.01	0.19
		TC	11 310	402	0.88 (0.74–1.04)		
		CC	3358	99	0.71 (0.54–0.93)		
		Per minor allele	27 169	994	0.85 (0.76–0.96)		
<i>XPC</i>	rs2228001	AA	9514	392	1.00 (ref)	0.002	0.11
		CA	13 049	477	0.89 (0.75–1.06) ⁴		
		CC	4796	130	0.67 (0.52–0.85) ⁴		
		Per minor allele	27 359	999	0.83 (0.74–0.94)		

Abbreviations: *AKR1C2*, aldo-keto reductase family 1 member C2; *CAT*, catalase; CI, confidence interval; FDR, false discovery rate; HR, hazard ratio; *HSD3B1/B2*, 3beta-hydroxysteroid dehydrogenase; *PTGS2*, prostaglandin-endoperoxide synthase 2; SNP, single nucleotide polymorphism; *XPC*, xeroderma pigmentosum, complementation group C.

¹Catalase (*CAT*) rs1001179 showed a positive association with advanced prostate cancer in the Netherlands Cohort Study on diet and cancer as previously reported by our group (ref. 34) and is therefore not presented here.

²Hazard ratios adjusted for age.

³P values adjusted for multiple testing comparisons using the false discovery rate (FDR) approach of Benjamini-Hochberg (1995); the FDR threshold was set at 0.20.

⁴Possible violation of the proportional hazards assumption but no statistically significant interaction with time (P value ≥ 0.05).

rs10923823 (*HSD3B1/B2*), and rs7546652 (*HSD3B1/B2*) showed an increase in risk for advanced prostate cancer, with HRs per minor allele of 1.16 (95% CI: (1.03–1.32); P trend = 0.02), 1.17 [95% CI: (1.05–1.31); P trend = 0.01], 1.14 [95% CI: (1.01–1.27); P trend = 0.03] and 1.14 [95% CI: (1.02–1.28); P trend = 0.03], respectively. A decreased risk of advanced prostate cancer was observed for men with variant alleles of rs5275 (*PTGS2*) and rs2228001 (*XPC*), with HRs per minor allele of 0.85 [95% CI: (0.76–0.96); P trend = 0.01] and 0.83 [95% CI: (0.74–0.94); P trend = 0.002], respectively. After multiple testing correction, rs511895 in *CAT* (FDR-adjusted P value = 0.15), rs5275 in *PTGS2* (FDR-adjusted P value = 0.19) and rs2228001 in *XPC* (FDR-adjusted P value = 0.11), remained significant at level 0.20. For the other SNPs and 2 gene deletions, we did not observe clear associations with advanced prostate cancer risk (data not shown).

Interactions between Acrylamide Intake and SNPs

Out of 58 analyzed SNPs and 2 gene deletions, two SNPs showed a nominally statistically significant multiplicative

interaction with acrylamide intake in the total study population (Table 4); rs1800566 (*NQO1*) with a P interaction of 0.03 and rs2301241 (*TXN*) with a P interaction of 0.04. Neither remained significant at level 0.20 after adjusting for multiple comparisons and we did not observe a clear dose-response relationship for acrylamide in strata of those genotypes. In non-smokers, no SNPs showed evidence of multiplicative interaction with acrylamide intake. A detailed overview of the acrylamide-gene interactions is provided in Supplementary Table S2.

In sensitivity analyses, we analyzed the acrylamide-gene interactions in non-smokers for 13.3 years of follow-up. Two SNPs (rs11252859 in *AKR1C1* and rs8192120 in *SRD5A1*) showed a nominally statistically significant multiplicative interaction with acrylamide intake, but they did not withstand correction for multiple testing (data not shown).

Discussion

In this large population-based prospective cohort study, we analyzed acrylamide-gene interactions for advanced prostate cancer risk, which has not been done before. Six

Table 4. SNPs showing nominally statistically significant interaction (P for interaction < 0.05) with dietary acrylamide on advanced prostate cancer risk after 20.3 years of follow-up; Netherlands Cohort Study on diet and cancer (1986–2006).

Gene	SNP	Study population	Genotype	Acrylamide, continuous		Acrylamide, quartiles of intake										P for interaction	FDR-adjusted P value ²
				n cases	total	10 µg/day		Q1		Q2		Q3		Q4			
						HR (95% CI) ¹	HR (95% CI) ¹	n cases	HR (95% CI) ¹	n cases	HR (95% CI) ¹	n cases	HR (95% CI) ¹	n cases	HR (95% CI) ¹		
NQO1	rs1800566	Total	CC	645		1.09 (1.00–1.19)	157	1.00 (ref)	159	1.02 (0.77–1.35)	151	1.03 (0.78–1.38)	178	1.17 (0.89–1.55)	0.22	0.03	0.96
			CT+TT	302		0.93 (0.82–1.06)	82	1.00 (ref)	79	0.87 (0.57–1.33)	73	0.74 (0.48–1.12)	68	0.70 (0.46–1.07)	0.14		
TXN	rs2301241	Total	TT	377		0.94 (0.83–1.05)	100	1.00 (ref)	94	0.90 (0.61–1.33)	89	0.79 (0.54–1.15)	94	0.76 (0.52–1.11)	0.16	0.04	0.96
			CT+CC	569		1.09 (1.01–1.19)	138	1.00 (ref)	144	1.02 (0.76–1.38)	134	1.04 (0.76–1.41)	153	1.20 (0.89–1.62)	0.19		

Abbreviations: CI, confidence interval; FDR, false discovery rate; HR, hazard ratio; NQO1, NAD(P)H quinone dehydrogenase 1; SNP, single nucleotide polymorphism; TXN, thioredoxin.
¹Hazard ratios adjusted for age, family history of prostate cancer, cigarette smoking status (never/former/current), frequency of smoking (number of cigarettes per day; centered), and duration of smoking (number of years; centered).
²P values adjusted for multiple testing comparisons using the false discovery rate (FDR) approach of Benjamini–Hochberg (1995); the FDR threshold was set at 0.20.

SNPs were associated with advanced prostate cancer risk, three of which remained significant after multiple comparisons correction (rs511895 in *CAT*, rs5275 in *PTGS2*, and rs2228001 in *XPC*). Two SNPs (rs1800566 in *NQO1* and rs2301241 in *TXN*) showed a nominally statistically significant multiplicative interaction with acrylamide intake, but neither remained significant after adjusting for multiple comparisons.

CAT is an antioxidant enzyme that plays a key role in oxidative stress protection by degrading hydrogen peroxide (35). The intronic *CAT* rs511895 SNP showed no association with lethal prostate cancer in the Health Professionals Follow-up Study (HPFS) (36). In the same study, this SNP was associated with circulating levels of alpha-tocopherol (Vitamin E), an antioxidant that may reduce prostate cancer risk. This may indicate that *CAT* rs511895 is a functional SNP or in linkage disequilibrium with another functional SNP. In the present study, participants with one or two variant alleles of *CAT* rs511895 had a higher advanced prostate cancer risk than homozygous wild type participants. In a previous study by our group (34), another *CAT* SNP (rs1001179) was also associated with increased advanced prostate cancer risk. As discussed by the authors, this association could be explained by reduced catalase activity and, consequently, deficiency in antioxidant protection against oxidative stress. We cannot provide such an explanation for *CAT* rs511895, which may indicate that our finding is due to chance (even after correction for multiple testing). *PTGS2* encodes COX-2, an enzyme that converts arachidonic acid to prostaglandin H₂ (37). COX-2 promotes inflammation and is overexpressed in various cancers, including prostate cancer (38). According to a meta-analysis (39), *PTGS2* rs5275 was not associated with prostate cancer risk in Caucasians, but in that study prostate cancer subtypes were not examined. In our study, the rare allele of *PTGS2* rs5275 was associated with a decreased advanced prostate cancer risk. *PTGS2* rs5275 is located in the 3'-untranslated region of the *PTGS2* gene and thought to regulate mRNA stability and degradation (40), thereby possibly influencing prostate cancer carcinogenesis. However, the *PTGS2* rs5275 SNP is not clearly associated with *PTGS2* gene expression in lymphoblastoid cell lines (41). *XPC* is a protein (encoded by the *XPC* gene) that plays an important role in DNA damage recognition, in the global genome nucleotide excision repair (GG-NER) pathway (42). A meta-analysis found that the non-synonymous coding *XPC* rs2228001 SNP was not associated with prostate cancer risk in Caucasians (43). However, only two studies were included (including one study with a small sample size) and prostate cancer subtypes were not examined. In our study, we show that the rare allele of *XPC* rs2228001 was

associated with a decreased advanced prostate cancer risk. Various studies, however, have shown that this polymorphism was associated with increased cancer risk through decreased DNA repair capacity (44). This indicates that the inverse association we found lacks biological plausibility, and may therefore represent a chance finding. With regard to all three SNPs (rs511895 in *CAT*, rs5275 in *PTGS2*, and rs2228001 in *XPC*), future well-designed gene-association studies with large sample size are required to confirm our findings.

Prior to acrylamide-gene interaction analysis, we examined the association between dietary acrylamide and advanced prostate cancer risk after 20.3 years of follow-up. The (statistically non-significant) inverse association we observed across quintiles of acrylamide intake and advanced prostate cancer risk in never-smokers after 13.3 years of follow-up (8) did not persist after 20.3 years of follow-up. Thus, the previously observed inverse association may have been due to chance since the association was not statistically significant. In our earlier study, we interpreted the putative inverse association in the context of the associations we found with other hormone-related cancers. A Swedish prospective study (9), conducted after our study, also reported an (statistically non-significant) inverse association between acrylamide intake and advanced prostate cancer risk in never-smokers. However, the third other study (10) did not find an association. Given this limited and inconsistent evidence, we examined acrylamide-gene interactions in order to better understand a possible association between acrylamide intake and prostate cancer. For that matter, we selected genetic variants involved in acrylamide metabolism and the hypothesized mechanisms of acrylamide-induced carcinogenesis: genotoxicity (DNA repair), a sex hormonal effect and oxidative stress (11). While we observed two SNPs (rs1800566 in *NQO1* and rs2301241 in *TXN*) that showed statistically significant multiplicative interaction in the total study population, we did not identify SNPs that survived multiple testing correction or multiple SNPs in the same gene or SNPs that showed a clear dose-response relationship for acrylamide in strata of the genotypes. Thus, the current study does not provide evidence for an interaction between selected genetic variants and acrylamide intake on advanced prostate cancer risk. Consequently, this study does not increase the strength of evidence for a causal association between acrylamide intake and prostate cancer risk.

Preferably, we would have performed acrylamide-gene interactions in never-smokers to eliminate any confounding effects by smoking (which is an important source of acrylamide) and to be able to shed more light on the previously reported inverse association for this group. However, the number of available cases in this

group was too small for this purpose and therefore we combined never-smokers with former smokers who had quit smoking more than 10 years before baseline. Unpublished results by our group did not show an association between former smoking and advanced prostate cancer risk and other studies reported mixed results (31,32), which made us decide to combine these two groups. Of course, residual confounding by former smoking may still have been present, but we tried to eliminate this as much as possible by detailed adjustment for (former) smoking. It is therefore not to be expected that analyzing this non-smoking group has rendered importantly different results than analyzing never-smokers would have done.

Strengths of our study are the prospective nature, the (>96%) completeness (45), and duration of cancer follow-up. A drawback of our study is the one-time baseline assessment of exposures and covariables. However, older people are likely to have relatively stable diets over time. Another drawback of our study is that we focused on functional candidate genes and variants associated with acrylamide metabolism and the hypothesized mechanisms of acrylamide-induced carcinogenesis. Therefore, we may have missed variants in genes that possibly interact with dietary acrylamide on advanced prostate cancer risk. Furthermore, even though we used data from a large cohort study, a relatively small number of cases per cell in acrylamide-gene analysis may have possibly resulted in limited statistical power to show statistically significant multiplicative interactions. Finally, baseline characteristics differed not significantly between participants who provided toenail clippings for DNA-analyses and participants who did not, except for level of education and cigarette smoking status. However, given the prospective cohort design of our study this is not likely to have biased our results.

In conclusion, the Netherlands Cohort Study on diet and cancer does not provide clear evidence for an interaction between acrylamide intake and selected genetic variants on advanced prostate cancer risk and does not increase the strength of evidence for a causal association between acrylamide intake and prostate cancer risk.

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Conflict of interest

Dr. Leo Schouten received compensation as a member of a scientific advisory panel on acrylamide risk assessment of the European Food Safety Authority. The other authors have no conflict of interests to declare.

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References

1. Brawer MK: Hormonal therapy for prostate cancer. *Rev Urol* 8(Suppl 2), S35–S47, 2006.
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, et al.: Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 136(5), E359–E386, 2015. doi:10.1002/ijc.29210.
3. Discacciati A, and Wolk A: Lifestyle and dietary factors in prostate cancer prevention. *Recent Results Cancer Res* 202, 27–37, 2014. doi:10.1007/978-3-642-45195-9_3.
4. Zhou CK, Check DP, Lortet-Tieulent J, Laversanne M, Jemal A, et al.: Prostate cancer incidence in 43 populations worldwide: an analysis of time trends overall and by age group. *Int J Cancer* 138(6), 1388–1400, 2016. doi:10.1002/ijc.29894.
5. Cook LS, Goldoft M, Schwartz SM, and Weiss NS: Incidence of adenocarcinoma of the prostate in Asian immigrants to the United States and their descendants. *J Urol* 161, 152–155, 1999.
6. Kimura T: East meets West: ethnic differences in prostate cancer epidemiology between East Asians and Caucasians. *Chin J Cancer* 31(9), 421–429, 2012. doi:10.5732/cjc.011.10324.
7. Pelucchi C, Bosetti C, Galeone C, and La Vecchia C: Dietary acrylamide and cancer risk: an updated meta-analysis. *Int J Cancer* 136(12), 2912–2922, 2015. doi:10.1002/ijc.29339.
8. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, and van den Brandt PA: Dietary acrylamide intake and the risk of renal cell, bladder, and prostate cancer. *Am J Clin Nutr* 87, 1428–1438, 2008.
9. Larsson SC, Akesson A, and Wolk A: Dietary acrylamide intake and prostate cancer risk in a prospective cohort of

- Swedish men. *Cancer Epidemiol Biomarkers Prev* **18**(6), 1939–1941, 2009. doi:10.1158/1055-9965.epi-09-0280.
10. Wilson KM, Giovannucci E, Stampfer MJ, and Mucci LA: Dietary acrylamide and risk of prostate cancer. *Int J Cancer* **131**(2), 479–487, 2012. doi:10.1002/ijc.26383.
 11. Besaratinia A, and Pfeifer GP: A review of mechanisms of acrylamide carcinogenicity. *Carcinogenesis* **28**(3), 519–528, 2007. doi:10.1093/carcin/bgm006.
 12. Besaratinia A, and Pfeifer GP: Genotoxicity of acrylamide and glycidamide. *J Natl Cancer Inst* **96**, 1023–1029, 2004.
 13. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, and van den Brandt PA: A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev* **16**(11), 2304–2313, 2007. doi:10.1158/1055-9965.epi-07-0581.
 14. Reuter S, Gupta SC, Chaturvedi MM, and Aggarwal BB: Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med* **49**(11), 1603–1616, 2010. doi:10.1016/j.freeradbiomed.2010.09.006.
 15. Finkel T, and Holbrook NJ: Oxidants, oxidative stress and the biology of ageing. *Nature* **408**, 239–247, 2000. doi:10.1038/35041687.
 16. Khandrika L, Kumar B, Koul S, Maroni P, and Koul HK: Oxidative stress in prostate cancer. *Cancer Lett* **282**(2), 125–136, 2009. doi:10.1016/j.canlet.2008.12.011.
 17. Kulathinal S, Karvanen J, Saarela O, and Kuulasmaa K: Case-cohort design in practice – experiences from the MORGAM Project. *Epidemiol Perspect Innov* **4**, 15, 2007. doi:10.1186/1742-5573-4-15.
 18. Van den Brandt PA, Schouten LJ, Goldbohm RA, Dorant E, and Hunen PM: Development of a record linkage protocol for use in the Dutch Cancer Registry for Epidemiological Research. *Int J Epidemiol* **19**, 553–558, 1990.
 19. van den Brandt PA, Goldbohm RA, van 't Veer P, Volovics A, Hermus RJ, et al.: A large-scale prospective cohort study on diet and cancer in The Netherlands. *J Clin Epidemiol* **43**, 285–295, 1990.
 20. Sobin LH, Gospodarowicz MK, and Wittekind C: TNM Classification of Malignant Tumors, 7th ed. Oxford, UK: Wiley-Blackwell; 2009.
 21. Goldbohm RA, van den Brandt PA, Brants HA, van't Veer P, Al M, et al.: Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. *Eur J Clin Nutr* **48**, 253–265, 1994.
 22. Goldbohm RA, van't Veer P, van den Brandt PA, van't Hof MA, Brants HA, et al.: Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. *Eur J Clin Nutr* **49**, 420–429, 1995.
 23. Konings EJ, Hogervorst JG, van Rooij L, Schouten LJ, Sizoo EA, et al.: Validation of a database on acrylamide for use in epidemiological studies. *Eur J Clin Nutr* **64**, 534–540, 2010. doi:10.1038/ejcn.2010.17.
 24. Hogervorst JG, van den Brandt PA, Godschalk RW, van Schooten FJ, and Schouten LJ: The influence of single nucleotide polymorphisms on the association between dietary acrylamide intake and endometrial cancer risk. *Sci Rep* **6**(34902), 2016. doi:10.1038/srep34902.
 25. Huang YF, Chen ML, Liou SH, Chen MF, Uang SN, et al.: Association of CYP2E1, GST and mEH genetic polymorphisms with urinary acrylamide metabolites in workers exposed to acrylamide. *Toxicol Lett* **203**(2), 118–126, 2011. doi:10.1016/j.toxlet.2011.03.008.
 26. Hogervorst JG, Godschalk RW, van den Brandt PA, Weijenberg MP, Verhage BA, et al.: DNA from nails for genetic analyses in large-scale epidemiologic studies. *Cancer Epidemiol Biomarkers Prev* **23**(12), 2703–2712, 2014. doi:10.1158/1055-9965.epi-14-0552.
 27. Gabriel S, Ziaugra L, and Tabbaa D: SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr Protoc Hum Genet* **Chapter 2**, Unit 2.12, 2009. doi:10.1002/0471142905.hg0212s60.
 28. Benjamini Y, and Hochberg Y: Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B (Methodological)* **57**, 289–300, 1995.
 29. Lin DY, and Wei LJ: The robust inference for the cox proportional hazards model. *J Am Stat Assoc* **84**(408), 1074–1078, 1989. doi:10.2307/2290085.
 30. Schoenfeld D: Partial residuals for the proportional hazards regression model. *Biometrika* **69**(1), 239–241, 1982. doi:10.2307/2335876.
 31. Huncharek M, Haddock KS, Reid R, and Kupelnick B: Smoking as a risk factor for prostate cancer: a meta-analysis of 24 prospective cohort studies. *Am J Public Health* **100**(4), 693–701, 2010. doi:10.2105/ajph.2008.150508.
 32. Islami F, Moreira DM, Boffetta P, and Freedland SJ: A systematic review and meta-analysis of tobacco use and prostate cancer mortality and incidence in prospective cohort studies. *Eur Urol* **66**(6), 1054–1064, 2014. doi:10.1016/j.eururo.2014.08.059.
 33. Geybels MS, van den Brandt PA, Schouten LJ, van Schooten FJ, van Breda SG, et al.: Selenoprotein gene variants, toenail selenium levels, and risk for advanced prostate cancer. *J Natl Cancer Inst* **106**, dju003, 2014. doi:10.1093/jnci/dju003.
 34. Geybels MS, van den Brandt PA, van Schooten FJ, and Verhage BA: Oxidative stress-related genetic variants, pro- and antioxidant intake and status, and advanced prostate cancer risk. *Cancer Epidemiol Biomarkers Prev* **24**(1), 178–186, 2015. doi:10.1158/1055-9965.epi-14-0968.
 35. Liou GY, and Storz P: Reactive oxygen species in cancer. *Free Radic Res* **44**(5), 479–496, 2010. doi:10.3109/10715761003667554.
 36. Van Blarigan EL, Ma J, Kenfield SA, Stampfer MJ, Sesso HD, et al.: Plasma antioxidants, genetic variation in SOD2, CAT, GPX1, GPX4, and prostate cancer survival. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **23**, 1037–1046, 2014. doi:10.1158/1055-9965.EPI-13-0670.
 37. Stack E, and DuBois RN: Regulation of cyclo-oxygenase-2. *Best Pract Res Clin Gastroenterol* **15**(5), 787–800, 2001. doi:10.1053/bega.2001.0235.
 38. Uotila P, Valve E, Martikainen P, Nevalainen M, Nurmi M, et al.: Increased expression of cyclooxygenase-2 and nitric oxide synthase-2 in human prostate cancer. *Urol Res* **29**(1), 23–28, 2001.
 39. Yang X, Li B, Si T, Liu Y, and Guo Z: Association between the 8473T>C polymorphism of PTGS2 and prostate cancer risk: a metaanalysis including 24,716 subjects. *Onkologie* **36**(4), 182–186, 2013. doi:10.1159/000349951.
 40. Hu Z, Miao X, Ma H, Wang X, Tan W, et al.: A common polymorphism in the 3'UTR of cyclooxygenase 2/

- prostaglandin synthase 2 gene and risk of lung cancer in a Chinese population. *Lung Cancer* **48**(1), 11–17, 2005. doi:10.1016/j.lungcan.2004.09.004.
41. Yang H, Gu J, Lin X, Grossman HB, Ye Y, et al.: Profiling of genetic variations in inflammation pathway genes in relation to bladder cancer predisposition. *Clin Cancer Res* **14**(7), 2236–2244, 2008. doi:10.1158/1078-0432.ccr-07-1670.
 42. Melis JP, Luijten M, Mullenders LH, and van Steeg H: The role of XPC: implications in cancer and oxidative DNA damage. *Mutat Res* **728**(3), 107–117, 2011. doi:10.1016/j.mrrev.2011.07.001.
 43. Chen Y, Zhong H, Gao JG, Tang JE, and Wang R: A systematic review and meta-analysis of three gene variants association with risk of prostate cancer: an update. *Urol J* **12**(3), 2138–2147, 2015.
 44. Francisco G, Menezes PR, Eluf-Neto J, and Chammas R: XPC polymorphisms play a role in tissue-specific carcinogenesis: a meta-analysis. *Eur J Hum Genet* **16**(6), 724–734, 2008. doi:10.1038/ejhg.2008.6.
 45. Goldbohm RA, van den Brandt PA, and Dorant E: Estimation of the coverage of Dutch municipalities by cancer registries and PALGA based on hospital discharge data. *Tijdschr Soc Gezondheidsz* **72**, 80–84, 1994.

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Acrylamide Hemoglobin Adduct Levels and Ovarian Cancer Risk: a nested case-control study

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Abstract

Background—Acrylamide is a probable human carcinogen formed during cooking of starchy foods. Two large prospective cohort studies of dietary acrylamide intake and ovarian cancer risk observed a positive association, although two other studies reported no association.

Methods—We measured acrylamide exposure using red blood cell acrylamide and glycidamide hemoglobin adducts among women in two large prospective cohorts: the Nurses' Health Study and Nurses' Health Study II. Between blood collection and 2010, we identified 263 incident cases of epithelial ovarian cancer, matching two controls per case. We used logistic regression models to examine the association between acrylamide exposure and ovarian cancer risk, adjusting for matching factors, family history of ovarian cancer, tubal ligation, oral contraceptive use, body mass index (BMI), parity, alcohol intake, smoking, physical activity, and caffeine intake.

Results—The multivariate-adjusted relative risk (RR) of ovarian cancer comparing the highest versus lowest tertile of total acrylamide adducts was 0.79 (95% CI: 0.50–1.24, *P* trend = 0.08). The comparable RR of ovarian cancer among non-smokers at blood draw was 0.85 (95% CI: 0.57–1.27, *P* trend = 0.14). The association did not differ by tumor histology (serous invasive versus not), *P* for heterogeneity = 0.41. Individual adduct types (acrylamide or glycidamide) were not associated with risk.

Conclusions—We observed no evidence that acrylamide exposure as measured by adducts to hemoglobin is associated with an increased risk of ovarian cancer.

Impact—Our finding indicates that acrylamide intake may not increase risk of ovarian cancer.

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Keywords

acrylamide; ovarian cancer; hemoglobin adducts; epidemiology; prospective

Introduction

Acrylamide is a chemical compound that forms naturally during the cooking of starchy foods (1). Moderate levels of acrylamide (5–50 mg/kg) have been found in heated protein-rich foods and higher levels (150–4000 mg/kg) in carbohydrate-rich foods, such as French fries, potato chips, baked goods, and cold breakfast cereal (2). Coffee also is an important source of acrylamide, which is formed during roasting of the beans. High levels also are found in cigarette smoke (3–5). In addition, small exposures to acrylamide could occur through drinking water, cosmetics, and passive smoking (6, 7).

Acrylamide was classified as “probably carcinogenic to humans” on the basis of positive cancer bioassay results by International Agency for Research on Cancer in 1994 (8). Evidence included that acrylamide was efficiently biotransformed to a chemically reactive genotoxic metabolite (glycidamide) in both rodents and humans (9), and in rodents this compound led to the development of certain cancers, particularly hormone-sensitive cancers (10, 11). In addition to genotoxic effects, acrylamide may bind to proteins related to maintenance of hormonal balance including human estrogen receptor (ER) and sex hormone-binding globulin (SHBG) (9).

Prior to 2002, acrylamide was thought to be mainly an industrial exposure; upon discovery of its formation in foods during cooking, many efforts have been made to estimate dietary exposures and to study whether this level of exposure is associated with cancer risk in human populations. Daily intake of acrylamide may be as high as a few tens of micrograms in western populations (12–14). Studies of acrylamide intake, assessed by food frequency questionnaire (FFQ) and risk of cancer at a variety of sites have been carried out since 2002, with generally null results (9, 15–27).

However, two large prospective studies observed positive associations between dietary acrylamide intake and risk of ovarian cancer (12, 28), though one other prospective study and a hospital-based case-control study observed no association (13, 14). Given the hypothesis that acrylamide may affect sex hormones, the positive findings for ovarian cancer are of particular interest. To further examine this association, we used a biomarker of exposure, acrylamide and glycidamide adducts to hemoglobin, to conduct a nested case-control study assessing the relationship between acrylamide exposure and ovarian cancer risk within the prospective Nurses’ Health Study (NHS) and NHSII cohorts.

Methods

Study Population

The NHS is a prospective cohort study established in 1976, when 121,700 U.S. female registered nurses, 30 to 55 years of age, completed an initial mailed questionnaire about their lifestyle factors, behaviors, and medical history. The NHSII is a prospective cohort established in 1989, when 116,430 U.S. female nurses, 25 to 42 years of age completed an initial questionnaire. Both cohorts have been followed by biennially mailed questionnaires to update exposure information, lifestyle factors, and ascertain non-fatal incident diseases since establishment. Women completed a semi-quantitative FFQ every 2–4 years since 1980 and 1991 in the NHS and NHSII, respectively. In the current study, we used dietary exposures from the 1990 FFQ for NHS and 1999 FFQ for NHS II, since they were the

closest to the time of blood collection for each cohort. Deaths in both cohorts were reported by family or postal authorities. We also searched for names of non-responders in the National Death Index (29, 30).

Between 1989 and 1990, 32,826 NHS participants (aged 43–70) provided blood samples with a short questionnaire (31). Similarly, from 1996 to 1999, 29,611 NHS II participants (aged 32–54) provided blood samples and a short questionnaire (32). In brief, women in both sub-cohorts had their blood drawn and shipped overnight on ice to our laboratory, where the blood was processed 24–36 hours after collection, and separated into plasma, red blood cells, and white blood cells. Samples were stored in liquid nitrogen freezers after collection. To assess the impact of the delay in processing on acrylamide adducts, we compared acrylamide adduct levels in blood processed immediately versus 24 or 48 hours after collection (N=12). The correlations for delayed versus immediate processing were > 0.98 for acrylamide and glycidamide, suggesting that these adducts are stable with delayed processing.

Measurement of exposure and laboratory assays

To measure hemoglobin adducts of acrylamide and glycidamide, erythrocytes were sent to the Protein Biomarker Laboratory, Clinical Chemistry Branch, Centers for Disease Control and Prevention, Atlanta, GA. Measurements were automated using the optimized Edman reaction described by Vesper et al using HPLC-MS/MS (33). The reaction products of acrylamide and glycidamide with the N-terminal valine of the hemoglobin protein chains were measured as N-[2-carbamoyl-ethyl]valine-pentafluorophenylhydantion (PFPTH) derivative and N-[2-hydroxycarbamoyl-ethyl]valine-pentafluorophenylhydantion (PFPTH) derivative for acrylamide and glycidamide adducts, respectively, and results were reported as pmol adduct per gram of total hemoglobin. Total hemoglobin concentration was measured from cyanmethemoglobin, which is formed from methemoglobin by reaction with cyanide (CMH solution) with the manufacturer's assay kit. The resulting red colored complex has peak absorption at 540 nm. Details of the assay have been published previously (34, 35).

Laboratory personnel were blinded to case status and matched cases and controls were assayed in the same batches. The inter-batch coefficient of variation from masked replicate samples in each batch were 8.7% for acrylamide adducts and 11.9% for glycidamide adducts. Samples were processed in 2 batches.

Assessment of covariates

Information on covariates was collected from the biennial questionnaires and the questionnaire completed at blood collection. Participants provided information on height at the cohort baseline. Family history of ovarian cancer was ascertained in 1992 (NHS) and 2001 (NHSII). Tubal ligation was assessed in 1994 and 1997, respectively. Alcohol and caffeine intake were measured in 1990 (NHS) and 1999 (NHSII) on the FFQ. Smoking and weight was ascertained at the time of blood collection in both cohorts. Physical activity was measured in 1992 (NHS) and 1997 (NHSII) respectively. Parity, age at first birth, menopausal status, post-menopausal hormone use, and oral contraceptive use were assessed biennially. For covariates with multiple measurements, we used information from the questionnaire completed closest to the date of blood collection.

Identification of ovarian cancer cases

We identified incident ovarian cancer cases by self-report on biennial questionnaires, reports from family members, the National Death Index, and the US Postal Service. For reported ovarian cancer cases, we obtained medical records or records from cancer registries for

confirmation. We confirmed 263 ovarian cancer cases diagnosed after blood collection but before 1 June 2010 for NHS and 1 June 2009 for NHSII. Cases were matched to two controls on age at blood draw, time of day of blood draw, month of blood draw, fasting status, menopausal status at blood draw and diagnosis, and postmenopausal hormone use at blood draw. Although smoking is an important source of acrylamide exposure, we did not match for this factor in the nested case-control study as it is not a strong risk factor for ovarian cancer; however, we carefully considered smoking in the statistical analysis (described below). The Institutional Review Board of the Brigham and Women's Hospital approved this analysis, and all participants provided informed consent.

Statistical Analyses

We categorized three continuous exposure variables, including total acrylamide (sum of acrylamide and glycidamide adducts) as well as the individual acrylamide, and glycidamide adducts, into tertiles based on the exposure distributions in controls. We used conditional logistic regression models to estimate relative risks (RR) and 95% confidence intervals (CI) of ovarian cancer for each exposure tertile, conditioning on matching factors (and assay batch, since a case and its matched controls were assayed in the same batch) and adjusting for covariates including height (continuous), family history of ovarian cancer (yes, no), tubal ligation (yes, no), oral contraceptive (OC) use (never, <1 y, 1–5 y, 5+ y), BMI (continuous), parity (yes, no), number of children (continuous), average number of alcoholic drinks per week (continuous), smoking (never, past and <15y since quitting, past and ≥15y since quitting, current), physical activity (<18MET/week, ≥18MET/week), and caffeine intake (continuous). We also additionally considered adjustment for smoking intensity (pack-years of smoking) among current and ever smokers, but this was not included in the final model as it did not change the results.

To additionally control for smoking, we conducted analyses restricted to non-smokers (and secondarily in never smokers). To do this, we categorized exposures based on the distributions in controls who were non-smokers (or never smokers) at blood collection. We used unconditional logistic regression models adjusting for all the matching factors and the covariates mentioned above plus assay batch. To test potential effect modification by menopausal status (pre- vs. postmenopausal), postmenopausal hormone use at blood draw (yes vs. no) among postmenopausal women, age (<55 vs. ≥55 years), and BMI (<25 vs. ≥25) we included multiplicative interaction terms in multivariate models and assessed statistical significance using the Likelihood ratio tests.

We used linear trend tests to examine possible trends across natural log-transformed continuous adduct levels and obtained trend p-values using the Wald statistic. We used polytomous logistic regression models to analyze whether the associations between exposures and ovarian cancer were different by histologic subtype (serous invasive versus other). P-values for interaction were obtained using likelihood ratio tests, comparing models allowing the associations with the exposure variable of interest to vary versus models not allowing the association to vary (36); we allowed age, parity, and smoking to vary in both models as these factors may be differently associated by histology (36).

We did not identify any statistical outliers using the generalized extreme studentized deviate many-outlier detection approach (37). We also assigned half the value of the limit of detection to any samples with values less than limit of detection (n=12 for glycidamide). We used SAS 9.3 software (SAS Institute, Cary, NC, USA) or STATA 12.1 software (StataCorp. College Station, TX, USA) for all analyses and used two-sided *P* values.

Results

We confirmed 263 incident cases of epithelial ovarian cancer during follow-up. The median interval between blood collection and diagnosis was 9.9 years. Cases were slightly more likely than controls to be current smokers (12.6% vs. 10.7%, respectively) or past smokers (39.9% vs. 38.1%), and had a higher prevalence of family history of ovarian cancer (6.8% vs. 2.1%) (Table 1). Controls were more likely than cases to be parous (93.2% vs. 88.2%, respectively), have more pregnancies (3.3 vs. 3.0), and have had a tubal ligation (20.6% vs. 16.4%). Median acrylamide and glycidamide adduct levels were 112.6 pmol/g Hb among cases and 113.9 pmol/g Hb among controls. Median levels in current smokers at blood draw (n=88) were 276.5 pmol/g Hb, compared to 108 pmol/g Hb among non-smokers (n=690). There were 79 serous cases versus 156 non-serous cases, 217 invasive cases versus 46 in-situ cases, and 129 cases diagnosed within 10 years versus 134 cases diagnosed more than 10 years.

Overall, we did not observe a statistically significant association between total acrylamide (i.e., the sum of acrylamide and glycidamide adducts) hemoglobin adducts and ovarian cancer risk (Table 2). Compared to women with total adduct concentrations of ≤ 99 pmol/g, the RRs were 0.83 (95% CI: 0.56, 1.24) for women with >99 –134.1 pmol/g and 0.79 (95% CI: 0.50, 1.24) for women with >134.1 pmol/g (P trend=0.08) (Table 2). When adducts were analyzed separately, no positive association was observed for glycidamide adducts (P trend=0.19), and a suggestive inverse association was noted for acrylamide adducts (P trend=0.05). Results from age-adjusted models were similar to those from multivariate-adjusted models (data not shown).

Since tobacco smoking is an important source of acrylamide exposure, we conducted an analysis restricted to women who were not current smokers at blood draw. Again, no associations were observed for total acrylamide adducts (P trend=0.13), acrylamide adducts (P trend=0.06), or glycidamide adducts (P trend=0.36). The comparable RRs were 0.84 (95% CI: 0.55, 1.27), 0.85 (95% CI: 0.56, 1.30), and 0.80 (95% CI: 0.52, 1.23), respectively. Results restricted to never smokers were similar to those among non-smokers (data not shown).

We assessed associations using quartiles of exposure, although the number of cases was small in each group. Most results were similar to main analyses, except the RR was 0.47 (95% CI: 0.26, 0.83) for women with acrylamide concentration >82.9 pmol/g (4th quartile) compared to women with ≤ 52.2 pmol/g (1st quartile). (Supplemental table) We also assessed associations comparing the top nine deciles of exposure to the bottom decile and no associations were observed (data not shown).

No differences in association were observed when restricting to invasive cases (data not shown). Similar results were observed for serous versus non-serous ovarian cancer cases, P for interaction=0.91, when considering cases diagnosed within 10 years from blood draw versus more than 10 years, P for interaction=0.79, and when removing caffeine intake from multivariate models (results not shown). No effect modification was observed for age, menopausal status at blood draw, PMH use at blood draw (p-values for interaction ≥ 0.30). For BMI at blood draw, there was a suggestion of an interaction with acrylamide (p-value for interaction=0.03). However, the confidence intervals were wide as there were 164 cases in the BMI <25 stratum and 99 cases in the BMI ≥ 25 stratum and there was no apparent trend of association in either strata (P trend=0.23 for women with BMI <25 and 0.58 for women with BMI ≥ 25).

Discussion

Ours is the first study to examine acrylamide hemoglobin adducts and risk of epithelial ovarian cancer. The median adducts levels measured in our study are in accordance with the range of previously published levels among smokers and non-smokers both in the US and Europe (4, 38–40). We did not observe an association between a biomarker of acrylamide intake, hemoglobin adducts of acrylamide and glycidamide, and ovarian cancer risk in a large nested case-control study of US women. Further, no associations were noted in non-smokers or never smokers or for serous invasive tumors.

Since tobacco use is a major source of acrylamide exposure, we carefully addressed possible confounding by smoking. Because the prevalence of smoking in this cohort is fairly low, we were able to restrict our analyses to non-smokers and secondarily never smokers at the time of blood collection, and noted no important differences in the acrylamide-ovarian cancer association. In addition, adjustment for pack-years of smoking did not alter the risk estimates. Finally, it is worth noting that smoking is only weakly associated with overall ovarian cancer risk and seems to be positively associated with only the mucinous type (41, 42); thus its role as a possible confounder is limited in spite of its very strong association with acrylamide adduct levels. However, passive smoking information, which could be a potential confounder, was not collected in our study.

The lack of association between acrylamide hemoglobin adducts and ovarian cancer risk in our study is in line with a prospective study and a hospital-based case-control study of FFQ-assessed dietary acrylamide intake and ovarian cancer (13, 14). Notably the prospective study did not have baseline information about smoking status, limiting the interpretation of the results. Conversely, two large prospective studies, including the NHS, reported positive associations for FFQ-assessed acrylamide. The Netherlands Cohort Study observed a RR comparing the highest versus lowest quintile of intake of 1.78 in all women (95% CI 1.10–2.88, P trend = 0.02) and 2.22 in never-smokers (95% CI 1.20–4.08, P trend = 0.01). In the NHS, higher dietary acrylamide intake was associated with an increased risk for serous ovarian cancer (comparable RR 1.58; 95% CI 0.99–2.52, P trend = 0.04) (12, 28). We did not observe an association with serous tumors in the current analysis of acrylamide adduct levels. It is not clear whether acrylamide exposure assessed by FFQ or by hemoglobin adducts is more biologically relevant for assessing ovarian cancer risk, thus it is important to consider the possible reasons for the differences in the observed associations.

These inconsistent findings between dietary studies may be because acrylamide comes from various sources of foods with variable concentrations that depend on cooking conditions as well as differences in study design. It is unclear why we previously observed an association for dietary acrylamide intake as measured through an FFQ in the NHS, but did not observe an association using a biomarker of acrylamide exposure. In our previous validation study, the correlation between dietary acrylamide intake and the sum of acrylamide hemoglobin adduct and glycidamide hemoglobin adduct was modest $r = 0.31$ (95% CI: 0.20–0.41) (43). In our current nested case-control study, we observed a similar correlation among non-smokers adjusting for batch, age, and energy intake of 0.25 (95% CI: 0.18–0.32). This modest correlation was confirmed in other studies as well (44–46). This suggests that while FFQs provide some information on acrylamide intake, there likely is notable measurement error associated with FFQ-assessed intake measures. This may be in part because acrylamide formation in food varies greatly depending on exact preparation and heating methods, or because FFQs do not capture all sources of exposure. Further, the moderately low correlation of dietary acrylamide with adducts levels in hemoglobin may in part be due to differences in how individuals absorb and metabolize acrylamide from food, including cytochrome P450 activity, which is not reflected in the dietary assessment (47, 48); thus the

two methods of exposure assessment are not fully comparable. Conversely, another possibility is that circulating acrylamide adducts levels do not adequately reflect exposure at the ovarian tissue level, since the ovarian epithelium has less vasculature than other organs (49).

However, the most plausible reason for the difference in associations observed in the FFQ versus our current study is that the variation in dietary acrylamide intake may be correlated with other lifestyle factors and health behaviors that are associated with an increased risk of ovarian cancer; that is, residual confounding may exist in the dietary studies. Strengths of our study include a relatively large number of cases and objective measurement of acrylamide exposure measured before ovarian cancer diagnosis. Power calculations indicated that the minimal detectable RR for 80% power in our study was 1.78, which is comparable to the associations observed in the dietary studies. In addition, we have prospective data on most known risk factors of ovarian cancer. We acknowledge that the exposure measurement relied on a single baseline measurement of acrylamide adducts in red blood cells and the follow-up was over a long time period, which may have induced non-differential misclassification and could potentially bias effect estimates toward the null. However, our previous work reported an intra-class correlation coefficient (ICC) of 0.77 over 3 years (43), suggesting that the between-person variation is much larger than the within-person variation and acrylamide measurement at one point in time appropriately ranks individuals with respect to their long-term exposure. In addition, no differences in associations were observed based on the timing of diagnosis in relation to blood draw. Due to very small number of cases within other histology subtypes, we had limited power to examine the association by tumor subtypes except for the serous versus non-serous tumors. Residual confounding may be of concern, although we comprehensively controlled or matched for major ovarian cancer risk factors and smoking.

Conclusions

Overall, this study does not support that acrylamide exposure, measured as hemoglobin adducts, is associated with an increased risk of ovarian cancer. Further studies with increased sample sizes and multiple blood measures are needed to confirm our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Tessier FJ, C N. The metabolic, nutritional and toxicological consequences of ingested dietary Maltol reaction products: a literature review. *J Soc Biol.* 2007; 201:199–207. [PubMed: 17978754]

2. Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M. Analysis of Acrylamide, a Carcinogen Formed in Heated Foodstuffs. *Journal of Agricultural and Food Chemistry*. 2002; 50:4998–5006. [PubMed: 12166997]
3. Kütting B, Uter W, Drexler H. The association between self-reported acrylamide intake and hemoglobin adducts as biomarkers of exposure. *Cancer Causes and Control*. 2008; 19:273–281. [PubMed: 17985202]
4. Wirfalt E, Paulsson B, Törnqvist M, Axmon A, Hagmar L. Associations between estimated acrylamide intakes, and hemoglobin AA adducts in a sample from the Malmo Diet and Cancer cohort. *Eur J Clin Nutr*. 2007; 62:314–323. [PubMed: 17356560]
5. Schettgen T, Rossbach B, Kütting B, Letzel S, Drexler H, Angerer J. Determination of haemoglobin adducts of acrylamide and glycidamide in smoking and non-smoking persons of the general population. *Int J Hyg Environ Health*. 2004; 207:531–539. [PubMed: 15729833]
6. Gargas ML, Kirman CR, Sweeney LM, Tardiff RG. Acrylamide: Consideration of species differences and nonlinear processes in estimating risk and safety for human ingestion. *Food and Chemical Toxicology*. 2009; 47:760–768. [PubMed: 19166901]
7. Amrein TM, Andres L, Escher F, Amadò R. Occurrence of acrylamide in selected foods and mitigation options. *Food Additives and Contaminants*. 2007; 24:13–25. [PubMed: 17687696]
8. IARC. Some industrial chemicals. *IARC Monogr Eval Carcinog Risks Hum*. 1994; 60:389–433. [PubMed: 7869577]
9. Hogervorst JGF, Baars B-J, Schouten LJ, Konings EJM, Goldbohm RA, van den Brandt PA. The carcinogenicity of dietary acrylamide intake: A comparative discussion of epidemiological and experimental animal research. *Critical Reviews in Toxicology*. 2010; 40:485–512. [PubMed: 20170357]
10. Johnson K, Gorzinski S, Bodner K, Campbell R, Wolf C, Friedman M, et al. Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol Appl Pharmacol*. 1986; 85:154–168. [PubMed: 3764902]
11. Friedman MA, Dulak LH, Stedham MA. A Lifetime Oncogenicity Study in Rats with Acrylamide. *Fundamental and Applied Toxicology*. 1995; 27:95–105. [PubMed: 7589934]
12. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. A Prospective Study of Dietary Acrylamide Intake and the Risk of Endometrial, Ovarian, and Breast Cancer. *Cancer Epidemiology Biomarkers & Prevention*. 2007; 16:2304–2313.
13. Larsson SC, Åkesson A, Wolk A. Long-Term Dietary Acrylamide Intake and Risk of Epithelial Ovarian Cancer in a Prospective Cohort of Swedish Women. *Cancer Epidemiology Biomarkers & Prevention*. 2009; 18:994–997.
14. Pelucchi C, Galeone C, Levi F, Negri E, Franceschi S, Talamini R, et al. Dietary acrylamide and human cancer. *International Journal of Cancer*. 2006; 118:467–471.
15. Wilson KM, Giovannucci E, Stampfer MJ, Mucci LA. Dietary acrylamide and risk of prostate cancer. *International Journal of Cancer*. 2012; 131:479–487.
16. Burley VJ, Greenwood DC, Hepworth SJ, Fraser LK, de Kok TM, van Breda SG, et al. Dietary acrylamide intake and risk of breast cancer in the UK women's cohort. *Br J Cancer*. 2010; 103:1749–1754. [PubMed: 20959829]
17. Larsson SC, Åkesson A, Wolk A. Dietary Acrylamide Intake and Prostate Cancer Risk in a Prospective Cohort of Swedish Men. *Cancer Epidemiology Biomarkers & Prevention*. 2009; 18:1939–1941.
18. Hogervorst JGF, Schouten LJ, Konings EJM, Goldbohm RA, van den Brandt PA. Lung Cancer Risk in Relation to Dietary Acrylamide Intake. *Journal of the National Cancer Institute*. 2009; 101:651–662. [PubMed: 19401552]
19. Hogervorst JGF, Schouten LJ, Konings EJM, Goldbohm RA, van den Brandt PA. Dietary Acrylamide Intake and Brain Cancer Risk. *Cancer Epidemiology Biomarkers & Prevention*. 2009; 18:1663–1666.
20. Wilson KM, Mucci LA, Cho E, Hunter DJ, Chen WY, Willett WC. Dietary Acrylamide Intake and Risk of Premenopausal Breast Cancer. *American Journal of Epidemiology*. 2009; 169:954–961. [PubMed: 19224978]

21. Larsson SC, Åkesson A, Bergkvist L, Wolk A. Dietary acrylamide intake and risk of colorectal cancer in a prospective cohort of men. *European Journal of Cancer*. 2009; 45:513–516. [PubMed: 19121931]
22. Larsson SC, Håkansson N, Åkesson A, Wolk A. Long-term dietary acrylamide intake and risk of endometrial cancer in a prospective cohort of Swedish women. *International Journal of Cancer*. 2009; 124:1196–1199.
23. Hogervorst JGF, Schouten LJ, Konings EJM, Goldbohm RA, van den Brandt PA. Dietary Acrylamide Intake Is Not Associated with Gastrointestinal Cancer Risk. *The Journal of Nutrition*. 2008; 138:2229–2236. [PubMed: 18936224]
24. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. Dietary acrylamide intake and the risk of renal cell, bladder, and prostate cancer. *The American Journal of Clinical Nutrition*. 2008; 87:1428–1438. [PubMed: 18469268]
25. Lipworth L, Sonderman JS, Tarone RE, McLaughlin JK. Review of epidemiologic studies of dietary acrylamide intake and the risk of cancer. *European Journal of Cancer Prevention*. 2012; 21:375–386. 10.1097/CEJ.0b013e3283529b64. [PubMed: 22495255]
26. Pelucchi C, La Vecchia C, Bosetti C, Boyle P, Boffetta P. Exposure to acrylamide and human cancer—a review and meta-analysis of epidemiologic studies. *Annals of Oncology*. 2011; 22:1487–1499. [PubMed: 21239401]
27. Mucci LA, Wilson KM. Acrylamide Intake through Diet and Human Cancer Risk. *Journal of Agricultural and Food Chemistry*. 2008; 56:6013–6019. [PubMed: 18624443]
28. Wilson KM, Mucci LA, Rosner BA, Willett WC. A Prospective Study on Dietary Acrylamide Intake and the Risk for Breast, Endometrial, and Ovarian Cancers. *Cancer Epidemiology Biomarkers & Prevention*. 2010; 19:2503–2515.
29. Stampfer MJ, Willett WC, Speizer FE, Dysert DC, Lipnick R, Rosner B, et al. Test of the National Death Index. *Am J Epidemiol*. 1984; 119
30. Rich-Edwards JW, Corsano KA, MJ S. Test of the National Death Index and Equifax Nationwide Death Search. *Am J Epidemiol*. 1994; 140
31. Hankinson SE, Willett WC, Manson JE, Hunter DJ, Colditz GA, Stampfer MJ, et al. Alcohol, Height, and Adiposity in Relation to Estrogen and Prolactin Levels in Postmenopausal Women. *Journal of the National Cancer Institute*. 1995; 87:1297–1302. [PubMed: 7658481]
32. Tworoger SS, Sluss P, Hankinson SE. Association between Plasma Prolactin Concentrations and Risk of Breast Cancer among Predominately Premenopausal Women. *Cancer Research*. 2006; 66:2476–2482. [PubMed: 16489055]
33. Vesper H, Ospina M, Meyers T, Ingham L, Smith A, Gray JG, et al. Automated method for measuring globin adducts of acrylamide and glycidamide at optimized Edman reaction conditions. *Rapid Communications in Mass Spectrometry*. 2006; 20:959–964. [PubMed: 16479554]
34. Vesper HW, Bernert JT, Ospina M, Meyers T, Ingham L, Smith A, et al. Assessment of the Relation between Biomarkers for Smoking and Biomarkers for Acrylamide Exposure in Humans. *Cancer Epidemiology Biomarkers & Prevention*. 2007; 16:2471–2478.
35. Vesper, H. Laboratory Procedure Manual: N-terminal hemoglobin adducts of acrylamide and glycidamide. 2008. [cited; Available from: www.cdc.gov/nchs/data/nhanes/nhanes_03_04/l06age_c_met.pdf]
36. Gates MA, Rosner BA, Hecht JL, Tworoger SS. Risk Factors for Epithelial Ovarian Cancer by Histologic Subtype. *American Journal of Epidemiology*. 2010; 171:45–53. [PubMed: 19910378]
37. Rosner B. Percentage points for a generalized ESD many-outlier procedure. *Technometrics*. 1983; 25:165–172.
38. Vesper HW, Caudill SP, Osterloh JD, Meyers T, Scott D, Myers GL. Exposure of the U.S. Population to Acrylamide in the National Health and Nutrition Examination Survey 2003–2004. *Environ Health Perspect*. 2010; 118:278–283. [PubMed: 20123601]
39. Wilson KM, Bälter K, Adami H-O, Grönberg H, Vikström AC, Paulsson B, et al. Acrylamide exposure measured by food frequency questionnaire and hemoglobin adduct levels and prostate cancer risk in the Cancer of the Prostate in Sweden Study. *International Journal of Cancer*. 2009; 124:2384–2390.

40. Thonning Olesen P, Olsen A, Frandsen H, Frederiksen K, Overvad K, Tjønneland A. Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study. *International Journal of Cancer*. 2008; 122:2094–2100.
41. Tworoger SS, Gertig DM, Gates MA, Hecht JL, Hankinson SE. Caffeine, alcohol, smoking, and the risk of incident epithelial ovarian cancer. *Cancer*. 2008; 112:1169–1177. [PubMed: 18213613]
42. Ovarian cancer and smoking: individual participant meta-analysis including 28 114 women with ovarian cancer from 51 epidemiological studies. *The Lancet Oncology*. 2012; 13:946–956. [PubMed: 22863523]
43. Wilson K, Vesper H, Tocco P, Sampson L, Rosén J, Hellenäs K-E, et al. Validation of a food frequency questionnaire measurement of dietary acrylamide intake using hemoglobin adducts of acrylamide and glycidamide. *Cancer Causes and Control*. 2009; 20:269–278. [PubMed: 18855107]
44. Tran NL, Barraj LM, Murphy MM, Bi X. Dietary Acrylamide Exposure and Hemoglobin Adducts – National Health and Nutrition Examination Survey (2003–04). *Food and Chemical Toxicology*. 2010; 48:3098–3108. [PubMed: 20696196]
45. Ferrari P, Freisling H, Duell E, Kaaks R, Lujan-Barroso L, Clavel-Chapelon F, et al. Challenges in estimating the validity of dietary acrylamide measurements. *Eur J Nutr*. 2012; 1–10.
46. Vikström AC, Warholm M, Paulsson B, Axmon A, Wirfält E, Törnqvist M. Hemoglobin adducts as a measure of variations in exposure to acrylamide in food and comparison to questionnaire data. *Food and Chemical Toxicology*. 2012; 50:2531–2539. [PubMed: 22525869]
47. Sumner SCJ, Fennell TR, Moore TA, Chanas B, Gonzalez F, Ghanayem BI. Role of Cytochrome P450 2E1 in the Metabolism of Acrylamide and Acrylonitrile in Mice. *Chemical Research in Toxicology*. 1999; 12:1110–1116. [PubMed: 10563837]
48. Fennell TR, Sumner SCJ, Snyder RW, Burgess J, Spicer R, Bridson WE, et al. Metabolism and Hemoglobin Adduct Formation of Acrylamide in Humans. *Toxicological Sciences*. 2005; 85:447–459. [PubMed: 15625188]
49. Redmer D, Reynolds L. Angiogenesis in the ovary. *Rev Reprod*. 1996; 1:182–192. [PubMed: 9414456]

Table 1

Selected characteristics of study participants in a nested case-control study of ovarian cancer in the NHS and NHSII

	Cases (N=263)	Controls (N=515)
Median (10–90 percentile)		
Total adducts (sum of acrylamide and glycidamide), pmol/g hemoglobin	112.6 (72.0–209.7)	113.9 (74.1–226)
Acrylamide adducts, pmol/g hemoglobin	63.8 (42.1–119)	62.2 (43.5–130)
Glycidamide adducts, pmol/g hemoglobin	49.5 (29.2–88.5)	51.1 (29.4–92.6)
Mean (SD)		
Age *, y	55.1 (7.5)	55.2 (7.5)
Height, m	1.65 (0.06)	1.64 (0.07)
BMI, kg/m ²	25.2 (5.3)	25.2 (4.5)
Duration of OC use among ever OC users, y	2.0 (3.3)	2.4 (3.6)
Parity among parous women, number	3.0 (1.4)	3.3 (1.5)
Alcohol intake, number of drinks/week	3.5 (4.8)	3.2 (4.4)
Physical activity, MET-hr/week	21.6 (22.0)	21.7 (22.8)
Caffeine intake, mg/day	244.4 (209.2)	251.5 (216.5)
Percentage		
Current smoker	12.6	10.7
Past smoker	39.9	38.1
Parous	88.2	93.2
Family history of ovarian cancer	6.8	2.1
Tubal ligation	16.4	20.6
Postmenopausal *	57.0	56.9
Postmenopausal hormone use among postmenopausal or unknown menopausal status women *	48.0	45.6
White race	99.2	99.6
Study participants from NHS	84.0	84.3

* Matching factor

Table 2

Multivariate-adjusted ovarian cancer RR (95% CI) by tertile of hemoglobin adducts of acrylamide and glycidamide in the NHS and NHSII

Total Adducts (acrylamide and glycidamide)					
All	Cases	T1	T2	T3	<i>P</i> _{trend}
Cases/controls		94/170	84/176	85/169	
Cut-points* (pmol/g Hb)		0-99	>99-134.1	>134.1	
RR [†]	263	1.00	0.83 (0.56-1.24)	0.79 (0.50-1.24)	0.08
Serous [‡]	156	1.00	0.93 (0.59-1.46)	0.82 (0.49-1.37)	<i>P</i> _{het} [°]
Non-serous [£]	79	1.00	0.96 (0.53-1.75)	0.69 (0.34-1.40)	0.86
Non-smokers					
Cases/controls		82/152	75/157	73/151	
Cut-points ^Φ (pmol/g Hb)		0-95.7	>95.8-124.2	>124.2	
RR [‡]	230	1.00	0.84 (0.56-1.26)	0.84 (0.55-1.27)	0.13
Acrylamide					
All	Cases	T1	T2	T3	<i>P</i> _{trend}
Cases/controls		92/171	84/175	87/169	
Cut-points* (pmol/g Hb)		0-54.6	>54.6-73.2	>73.2	
RR [†]	263	1.00	0.86 (0.58-1.26)	0.89 (0.57-1.39)	0.05
Serous [‡]	156	1.00	0.97 (0.62-1.54)	0.89 (0.54-1.49)	<i>P</i> _{het} [°]
Non-serous [£]	79	1.00	0.79 (0.42-1.46)	0.83 (0.42-1.63)	0.83
Non-smokers					
Cases/controls		80/152	75/157	75/151	
Cut-points ^Φ (pmol/g Hb)		0-52.3	>53.4-68.5	>68.5	
RR [‡]	230	1.00	0.87 (0.58-1.31)	0.85 (0.56-1.30)	0.06
Glycidamide					

All	T1	T2	T3	<i>P</i> trend [€]
Cases/controls	82/171	105/174	76/170	
Cut-points * (pmol/g Hb)	0-41.9	>41.9-61.6	>61.6	
RR [‡]	263 1.00	1.29 (0.88-1.90)	0.80 (0.49-1.29)	0.19
Serous [£]	156 1.00	1.08 (0.69-1.68)	0.71 (0.42-1.21)	<i>P</i> _{het} [°]
Non-serous [£]	79 1.00	1.72 (0.94-3.17)	0.87 (0.41-1.83)	0.37
Non-smokers				
Cases/controls	75/153	93/156	62/151	
Cut-points ^ϕ (pmol/g Hb)	0-40.2	>40.2-58	>58	
RR [‡]	230 1.00	1.14 (0.77-1.71)	0.80 (0.52-1.23)	0.36

[€] Linear trend test across exposures of log transformed interest using the Wald test determined the p-trend.

^{*} Cut-points obtained from controls

[‡] Conditioning on matching factors and adjusting for height, family history of ovarian cancer, tubal ligation, OC use, BMI, parous, number of additional children, average number of alcohol drinks per week, smoking, physical activity, and caffeine intake.

[£] Polytomous logistic regression adjusting for matching factors and the same covariates as listed above plus assay batch, allowing age at blood draw and parity to vary between serous and non-serous cases.

[°] Likelihood ratio test comparing full model (allowing the exposure association to vary by histology) to the reduced model that held exposure estimates constant across tumor subtype.

^ϕ Cut-points obtained from controls who were non-smokers

[‡] Unconditional logistic regression adjusting for matching factors and the same covariates listed above plus assay batch.

EXHIBIT 6

07/14/03 17:22 FAX 301 443 3100

FDA

002



DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Rockville MD 20857

Joan E. Denton, M.S., Ph.D.
Director
Office of Environmental Health Hazard Assessment
Proposition 65 Implementation
P.O. Box 4010
1001 I Street, 19th Floor
Sacramento, California 95812-4010

Dear Dr. Denton:

Under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65), California currently has a no significant risk level (NSRL) for acrylamide of .2 micrograms per day. We understand that California intends to announce a revised approach to acrylamide in the near future. FDA believes that it is premature to set a level for acrylamide in food, and that California's current NSRL and future actions may frustrate federal purposes or even directly conflict with federal law. More information is needed on the risks to humans from acrylamide in foods and on whether and how acrylamide levels in food can be safely reduced. FDA has created an extensive Action Plan (which is attached) outlining the steps FDA believes necessary to answer these questions. The Action Plan includes the following major goals, most of which relate to expanding the research base on acrylamide:

- Develop rapid or inexpensive screening methods and validate confirmatory methods of analysis.
- Identify mechanisms responsible for the formation of acrylamide in foods and identify means to reduce acrylamide exposure.
- Assess the dietary exposure of U.S. consumers to acrylamide by measuring acrylamide levels in various foods and estimating dietary exposure.
- Characterize the potential risks and uncertainties associated with exposure to acrylamide in foods by assessing the available information, by expanding research into acrylamide toxicology to reduce uncertainty, and by performing a quantitative risk assessment with the new information.
- Develop and foster public/private partnerships to gather scientific and technological information and data for assessing the human risk.
- Inform and educate consumers and processors about the potential risks associated with acrylamide throughout the assessment process and as knowledge is gained.
- Provide all the essential elements for risk analysis, i.e., risk assessment, risk communication, and risk management.

* See Tab 5

07/14/03 17:22 FAX 301 443 3100

FDA

003

Joan E. Denton, M.S., Ph.D.

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The FDA Food Advisory Committee, consisting of outside experts on food safety, has endorsed FDA's approach to acrylamide. Furthermore, the Food and Agriculture Organization (FAO) and World Health Organization (WHO) held a consultation on acrylamide on June 25-27, 2002, and did not suggest setting levels for acrylamide in food. The consultation concluded that the "information on the levels of acrylamide in food is far from complete." The consultation outlined needed research on acrylamide in foods, including methods of analysis for acrylamide, formation and fate of acrylamide in food, exposure assessment, non-cancer toxicology, genotoxicity, and carcinogenicity. The consultation also provided some advice to minimize whatever risk exists from acrylamide in foods, including avoiding excessive cooking of food (but cooking food thoroughly to destroy foodborne pathogens), choosing healthy eating, investigating possibilities for reducing levels of acrylamide in food, and establishing an international network on acrylamide in food to encourage sharing of data and ongoing investigations.

In addition, the Joint Expert Committee on Food Additives (JECFA), an international expert committee that evaluates food additives and contaminants for Codex Alimentarius, is scheduled to conduct a risk assessment on acrylamide in February 2005. Results of the JECFA risk assessment will be an invaluable part of a well-considered approach to any regulation of acrylamide in food.

Based on preliminary estimates provided by Grocery Manufacturers of America, many foods (including French fries, potato chips, cereals, breads, and coffee) might have to be labeled based on the present NSRL for acrylamide of 0.2 micrograms/day. FDA is concerned that premature labeling of many foods with warnings about dangerous levels of acrylamide would confuse and could potentially mislead consumers, both because the labeling would be so broad as to be meaningless and because the risk of consumption of acrylamide in food is not yet clear.

Furthermore, consumers may be misled into thinking that acrylamide is only a hazard in store-bought food. In fact, consumer exposure may be greatest through home cooking. Some of FDA's research will try to answer questions on the relationship between the degree of browning and acrylamide formation in home cooking. In addition, a requirement for warning labels on food might deter consumers from eating foods with such labels. Consumers who avoid eating some of these foods, such as breads and cereals, may encounter greater risks because they would have less fiber and other beneficial nutrients in their diets. For these reasons, premature labeling requirements would conflict with FDA's ongoing efforts to provide consumers with effective scientifically based risk communication to prevent disease and promote health.

In addition, any warning label requirements imposed under Proposition 65 might encourage manufacturers to take premature steps to remove acrylamide from food by introducing additives or changing cooking processes. Such steps could have unforeseen adverse consequences on public health if the consequences of these changes on the introduction of other health hazards are

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not scientifically and thoughtfully considered. Currently, not enough is known about acrylamide formation to identify safe, effective, and practical modifications to food processing techniques that will clearly prevent or reduce formation. Studies on formation and methods to reduce acrylamide are currently underway in many labs around the world including at FDA's National Center for Food Safety and Technology.

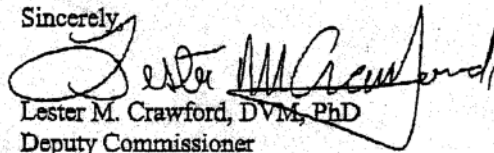
Also, California's current approach to acrylamide might discourage manufacturers from sharing data with FDA or with the Joint Institute for Food Safety and Applied Nutrition (JIFSAN), which is running the Acrylamide InfoNet for FAO/WHO. Such data would be helpful to FDA in its exposure and risk assessments for acrylamide.

FDA believes that California should not require warning labels for foods under Proposition 65 before completion of scientific studies adequate to assess the potential risk to consumers, as outlined in FDA's Action Plan, and until FDA determines appropriate risk management based on FDA's risk assessment. This approach will avoid confusing consumers and will assure that advice to consumers is scientifically founded. Although a precise time for the research and analysis cannot be predicted, it is expected to take 2-3 years.

Finally, FDA believes that California's current requirements for acrylamide under Proposition 65 and some actions that California may propose may be preempted by federal law to the extent that they frustrate federal purposes or create conflicts with federal law. For example, as discussed above, warning labels based on the presence of acrylamide in food might be misleading.

To ameliorate some of the concerns discussed above, California may wish to consider a regulatory approach for acrylamide which does not require warning labels on food. For example, Article 7, Section 12701, of the California Code of Regulations, "No Significant Risk Levels," defines the risk level which represents no significant risk as one that results in one excess cancer case per 100,000 population, with an exception applicable when "sound considerations of public health support an alternative level." The provision includes an example applicable "where chemicals in food are produced by cooking necessary to render the food palatable or to avoid microbiological contamination." California could designate acrylamide as a chemical "produced by cooking necessary to render the food palatable or to avoid microbiological contamination."

Sincerely,


Lester M. Crawford, DVM, PhD
Deputy Commissioner

Enclosure

cc: Mark B. McClellan, MD, PhD
Joseph A. Levitt, Esq.

EXHIBIT 7

EXHIBIT C



Acrylamide and Cancer Risk

What is acrylamide?

Acrylamide is a chemical used primarily to make substances called polyacrylamide and acrylamide copolymers. Polyacrylamide and acrylamide copolymers are used in many industrial processes, such as the production of paper, dyes, and plastics, and in the treatment of drinking water and wastewater, including sewage. They are also found in consumer products, such as caulking, food packaging, and some adhesives.

Acrylamide is also found in some foods. It can be produced when vegetables that contain the amino acid asparagine, such as potatoes, are heated to high temperatures in the presence of certain sugars (1, 2). It is also a component of tobacco smoke.

How are people exposed to acrylamide?

Food and cigarette smoke are the major sources of acrylamide exposure for people in the general population (3, 4).

The major food sources of acrylamide are French fries and potato chips; crackers, bread, and cookies; breakfast cereals; canned black olives; prune juice; and coffee.

Acrylamide levels in food vary widely depending on the manufacturer, the cooking time, and the method and temperature of the cooking process (5, 6). Decreasing cooking time to avoid heavy crisping or browning, blanching potatoes before frying, not storing potatoes in a refrigerator, and post-drying (drying in a hot air oven after frying) have been shown to decrease the acrylamide content of some foods (7, 8).

People are exposed to substantially more acrylamide from tobacco smoke than from food. People who smoke have three to five times higher levels of acrylamide exposure markers in their blood than do non-smokers (9). Exposure from other sources is likely to be significantly less than that from food or smoking, but scientists do not yet have a complete understanding of all sources of exposure. Regulations are in place to limit exposure in workplaces where acrylamide may be present, such as industrial settings that use polyacrylamide and acrylamide copolymers.

Is there an association between acrylamide and cancer?

Studies in rodent models have found that acrylamide exposure increases the risk for several types of cancer (10–13). In the body, acrylamide is converted to a compound called glycidamide, which causes mutations in and damage to DNA. However, a large number of epidemiologic studies (both case-control and cohort studies) in humans have found no consistent evidence that dietary acrylamide exposure is associated with the risk of any type of cancer (9, 14). One reason for the inconsistent findings from human studies may be the difficulty in determining a person's acrylamide intake based on their reported diet.

The [National Toxicology Program's Report on Carcinogens](#) considers acrylamide to be reasonably anticipated to be a human carcinogen, based on studies in laboratory animals given acrylamide in drinking water. However, toxicology studies have shown that humans and rodents not only absorb acrylamide at different rates, they metabolize it differently as well (15–17).

Studies of workplace exposure have shown that high levels of occupational acrylamide exposure (which occurs through inhalation) cause neurological damage, for example, among workers using acrylamide polymers to clarify water in coal preparation plants (18). However, studies of occupational exposure have not suggested increased risks of cancer (19).

Are acrylamide levels regulated?

The [U.S. Environmental Protection Agency \(EPA\) regulates acrylamide in drinking water](#). The EPA established an acceptable level of acrylamide exposure, set low enough to account for any uncertainty in the data relating acrylamide to cancer and neurotoxic effects. [The U.S. Food and Drug Administration regulates the amount of residual acrylamide](#) in a variety of materials that contact food, but there are currently no guidelines governing the presence of acrylamide in food itself.

What research is needed to better understand whether acrylamide is associated with cancer in people?

Additional epidemiologic studies in which acrylamide adduct or metabolite levels are serially measured in the same individuals over time (longitudinal cohorts) are needed to help determine whether dietary acrylamide intakes are associated with increased cancer risks in people. It is also important to determine how acrylamide is formed during the cooking process and whether acrylamide is present in foods other than those already tested. This information will enable researchers to make more accurate and comprehensive estimates of dietary exposure. Biospecimen collections in cohort studies will provide an opportunity to examine biomarkers of exposure to acrylamide and its metabolites in relation to the subsequent risk of cancer.

Where can people find additional information about acrylamide?

For more information about acrylamide in food, contact the FDA at 1-888-SAFEFOOD (1-888-723-3366) or visit their [Acrylamide](#) page.

Selected References

1. Stadler RH, Blank I, Varga N, et al. Acrylamide from Maillard reaction products. *Nature* 2002; 419(6906): 449–450. doi:[10.1038/419449a](https://doi.org/10.1038/419449a).

2. Mottram DS, Svednicka EL, Olsson KA. Acrylamide formed in the Maillard reaction. *Food Chem* 2002; 419(6906):448–449. doi:10.1038/419448a.
3. Urban M, Kavvadias D, Riedel K, Scherer G, Tricker AR. Urinary mercapturic acids and a hemoglobin adduct for the dosimetry of acrylamide exposure in smokers and nonsmokers. *Inhalation Toxicology* 2006; 18(10):831–839. [PubMed Abstract]
4. Çebi A. Acrylamide Intake, Its Effects on Tissue and Cancer. In: Gökmen V, editor. *Acrylamide in Food. Analysis, Content and Potential Health Effects*. London: Academic Press, 2016.
5. Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *Journal of Agricultural and Food Chemistry* 2002; 50(17):4998–5006. [PubMed Abstract]
6. Mojska H, Gielecinska I, Szponar L. Acrylamide content in heat-treated carbohydrate-rich foods in Poland. *Roczniki Panstwowego Zakladu Higieny* 2007; 58(1):345–349. [PubMed Abstract]
7. Kita A, Brathen E, Knutsen SH, Wicklund T. Effective ways of decreasing acrylamide content in potato crisps during processing. *Journal of Agricultural and Food Chemistry* 2004; 52(23):7011–7016. [PubMed Abstract]
8. Skog K, Viklund G, Olsson K, Sjöholm I. Acrylamide in home-prepared roasted potatoes. *Molecular Nutrition and Food Research* 2008; 52(3):307–312. [PubMed Abstract]
9. Virk-Baker MK, Nagy TR, Barnes S, Groopman J. Dietary acrylamide and human cancer: a systematic review of literature. *Nutrition and Cancer* 2014;66(5):774–790. [PubMed Abstract]
10. Dearfield KL, Abernathy CO, Ottley MS, Brantner JH, Hayes PF. Acrylamide: Its metabolism, developmental and reproductive effects, genotoxicity, and carcinogenicity. *Mutation Research* 1988; 195(1):45–77. [PubMed Abstract]
11. Dearfield KL, Douglas GR, Ehling UH, et al. Acrylamide: A review of its genotoxicity and an assessment of heritable genetic risk. *Mutation Research* 1995; 330(1–2):71–99. [PubMed Abstract]
12. Friedman M. Chemistry, biochemistry, and safety of acrylamide. A review. *Journal of Agricultural and Food Chemistry* 2003; 51(16):4504–4526. [PubMed Abstract]
13. National Toxicology Program. Toxicology and carcinogenesis studies of acrylamide (CASRN 79-06-1) in F344/N rats and B6C3F1 mice (feed and drinking water studies). *National Toxicology Program technical report series* 2012; (575):1–234. [PubMed Abstract]
14. Lipworth L, Sonderman JS, Tarone RE, McLaughlin JK. Review of epidemiologic studies of dietary acrylamide intake and the risk of cancer. *European Journal of Cancer Prevention* 2012; 21(4):375–386. [PubMed Abstract]
15. Fuhr U, Boettcher MI, Kinzig-Schippers M, et al. Toxicokinetics of acrylamide in humans after ingestion of a defined dose in a test meal to improve risk assessment for acrylamide carcinogenicity. *Cancer Epidemiology Biomarkers and Prevention* 2006; 15(2):266–271. [PubMed Abstract]
16. Fennell TR, Friedman MA. Comparison of acrylamide metabolism in humans and rodents. *Advances in experimental medicine and biology* 2005; 561:109–116. [PubMed Abstract]
17. Gargas ML, Kirman CR, Sweeney LM, Tardiff RG. Acrylamide: Consideration of species differences and nonlinear processes in estimating risk and safety for human ingestion. *Food and chemical toxicology* 2009; 47(4):760–768. [PubMed Abstract]
18. Mulloy KB. Two case reports of neurological disease in coal mine preparation plant workers. *American Journal of Industrial Medicine* 1996; 30(1):56–61. [PubMed Abstract]
19. Pelucchi C, La Vecchia C, Bosetti C, Boyle P, Boffetta P. Exposure to acrylamide and human cancer—a review and meta-analysis of epidemiologic studies. *Annals of Oncology* 2011; 22(7):1487–1499. [PubMed Abstract]

[Diet](#)[Harms of Cigarette Smoking and Health Benefits of Quitting](#)**Reviewed:** December 5, 2017

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EXHIBIT 8

EXHIBIT D



Acrylamide and Cancer Risk

What is acrylamide?

Acrylamide is a chemical used in industries such as the paper and pulp, construction, foundry, oil drilling, textiles, cosmetics, food processing, plastics, mining, and agricultural industries. It is used in making paper, dyes, and plastics, and in treating drinking water and wastewater.

Acrylamide can be found in small amounts in consumer products including caulk, food packaging, and some adhesives. It is also present in cigarette smoke.

Acrylamide can form naturally from chemical reactions in certain types of starchy foods, after cooking at high temperatures. Some foods with higher levels of acrylamide include French fries, potato chips, foods made from grains (such as breakfast cereals, cookies, and toast), and coffee.

Does acrylamide cause cancer?

In general, the American Cancer Society does not determine if something causes cancer (that is, if it is a carcinogen), but we do look to other respected organizations for help with this. Based on current research, some of these organizations have made the following determinations:

- The International Agency for Research on Cancer (IARC) (<https://www.iarc.fr/index.php>) classifies acrylamide as a **“probable human carcinogen.”**
- The US National Toxicology Program (NTP) (<https://ntp.niehs.nih.gov>) has classified acrylamide as **“reasonably anticipated to be a human carcinogen.”**
- The US Environmental Protection Agency (EPA) (<https://www.epa.gov>) classifies acrylamide as **“likely to be carcinogenic to humans.”**

It's important to note that these determinations are based mainly on studies in lab animals, and not on studies of people's exposure to acrylamide from foods. Since the discovery of acrylamide in foods in 2002, the American Cancer Society, the US Food and Drug Administration (FDA), the World Health Organization (WHO), the European Food

Safety Authority (EFSA), and many other organizations have recognized the need for further research on this topic. So far, reviews of studies done in groups of people (epidemiologic studies) suggest that dietary acrylamide isn't likely to be related to risk for most common types of cancer. But ongoing studies will continue to provide new information on whether acrylamide levels in foods are linked to increased cancer risk.

To learn more about how cancer causes are studied and classified, see [Known and Probable Human Carcinogens \(/cancer/cancer-causes/general-info/known-and-probable-human-carcinogens.html\)](#) and [Does This Cause Cancer? \(/cancer/cancer-causes/general-info/does-this-cause-cancer.html\)](#)

Are acrylamide levels regulated?

In the United States, the FDA regulates the amount of residual acrylamide in a variety of materials that come in contact with food, but there are currently no regulations on the presence of acrylamide in food itself. In 2016, the FDA issued guidance to help the food industry reduce the amount of acrylamide in certain foods, but these are recommendations, not regulations.

The EPA regulates acrylamide in drinking water. The EPA has set an acceptable level of acrylamide exposure, which is low enough to account for any uncertainty in the data relating acrylamide to cancer and other health effects.

In the workplace, exposure to acrylamide is regulated by the EPA and the Occupational Safety and Health Administration (OSHA).

Can acrylamide be avoided?

Some people working in certain industries that are regulated for acrylamide need to take precautions to limit their exposure.

For most people, the major potential sources of acrylamide exposure are in certain foods and in cigarette smoke. Avoiding cigarette smoke ([/healthy/stay-away-from-tobacco.html](#)) can lower your exposure to this and other harmful chemicals.

It's not yet clear if the levels of acrylamide in foods raise cancer risk, but if you're concerned, there are some things you can do to lower your exposure. In general, acrylamide levels rise when cooking is done for longer periods or at higher temperatures, and when certain types of cooking methods are used (such as frying or roasting). Here are some ways to reduce exposure to acrylamide in foods, according to the FDA:

- Limit foods that might be high in acrylamide, such as potato products (especially French fries and potato chips), coffee, and foods made from grains (such as

breakfast cereals, cookies, and toast.

- Limit certain cooking methods, such as frying and roasting, and limit the time certain foods are cooked. Boiling and steaming do not produce acrylamide.
- Soak raw potato slices in water for 15 to 30 minutes before frying or roasting to reduce acrylamide formation during cooking. (Soaked potatoes should be drained and blotted dry before cooking to prevent splattering or fires.)
- If frying potatoes or toasting bread, cook them to a lighter color (as opposed to dark brown), which produces less acrylamide.
- Avoid storing potatoes in the refrigerator, which can result in increased acrylamide levels during cooking.

To learn more

Along with the American Cancer Society, other sources of information about acrylamide include:

Food and Drug Administration

Acrylamide Questions and Answers:

www.fda.gov/Food/FoodborneIllnessContaminants/ChemicalContaminants/ucm053569.htm

(<http://www.fda.gov/Food/FoodborneIllnessContaminants/ChemicalContaminants/ucm053569.htm>)

National Cancer Institute

Acrylamide and Cancer Risk: www.cancer.gov/about-cancer/causes-prevention/risk/diet/acrylamide-fact-sheet (<http://www.cancer.gov/about-cancer/causes-prevention/risk/diet/acrylamide-fact-sheet>)

Agency for Toxic Substances and Disease Registry

ToxFAQs™ for Acrylamide: www.atsdr.cdc.gov/toxfaqs/tf.asp?id=1162&tid=236 (<http://www.atsdr.cdc.gov/toxfaqs/tf.asp?id=1162&tid=236>)

European Food Safety Authority (EFSA)

EFSA explains risk assessment: Acrylamide in food:

www.efsa.europa.eu/en/corporate/pub/acrylamide150604

(<http://www.efsa.europa.eu/en/corporate/pub/acrylamide150604>)

Written by References



The American Cancer Society medical and editorial content team
(/cancer/acs-medical-content-and-news-staff.html)

Our team is made up of doctors and oncology certified nurses with deep knowledge of cancer care as well as journalists, editors, and translators with extensive experience in medical writing.

Last Medical Review: February 11, 2019 | Last Revised: February 11, 2019

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MORE IN CANCER A-Z

Cancer Basics

Cancer Causes

Breast Cancer

Colon and Rectal Cancer

Skin Cancer

Lung Cancer

Prostate Cancer

View All Cancer Types

EXHIBIT 9

EXHIBIT F



Acrylamide

Why am I being warned about potential exposure to acrylamide?

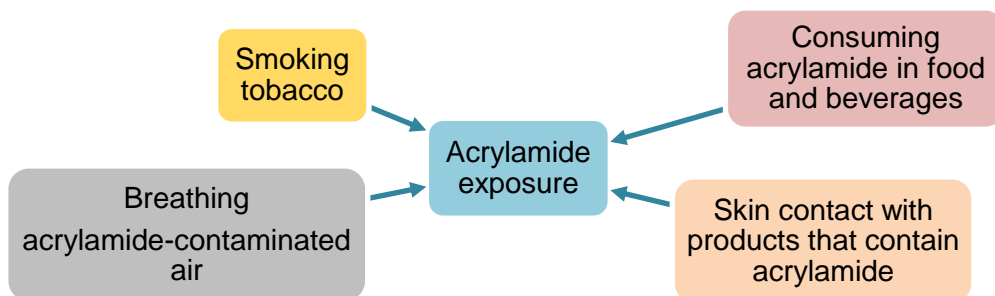


- Acrylamide is on the [Proposition 65](#) list because it can cause cancer. Exposure to acrylamide may increase the risk of cancer.
- Acrylamide is also on the Proposition 65 list because it can cause birth defects or other reproductive harm. It can affect the development of the fetus and can harm the male reproductive system. Levels in food are generally well below the levels currently believed to cause these harmful effects.
- Proposition 65 requires businesses to determine if they must provide a warning about exposures to [listed chemicals](#).

What is acrylamide?

- Acrylamide is a chemical that is formed in certain plant-based foods during cooking or processing at high temperatures, such as frying, roasting, grilling, and baking. Boiling and steaming foods do not create acrylamide.
 - ▶ Sources of acrylamide in the diet include French fries, potato chips, other fried and baked snack foods, roasted asparagus, canned sweet potatoes and pumpkin, canned black olives, roasted nuts, roasted grain-based coffee substitutes, prune juice, breakfast cereals, crackers, some cookies, bread crusts, and toast.
 - ▶ Researchers discovered the presence of acrylamide in fried, roasted and other cooked foods in 2002. High temperatures during cooking convert sugars and other naturally occurring substances in these foods to acrylamide.
- Tobacco smoke contains acrylamide.
- Acrylamide is used for industrial purposes. It has been used in grouts and cements. It is also used to produce polyacrylamide.

How does exposure to acrylamide occur?



- During pregnancy, acrylamide can pass from the mother to the baby.

How can I reduce my exposure to acrylamide?

- ❌ Do not smoke. Do not allow children to breathe tobacco smoke.
- ✓ The US Department of Health and Human Services recommends:
 - ▶ Adopt a healthy, balanced eating plan that includes fruits and vegetables, lean meats, fish, high-fiber grains and beans.
 - ▶ Fry foods at 170 degrees Celsius (338 degrees Fahrenheit) or lower temperatures. *[The higher the frying temperature, the more acrylamide is formed].*
 - *[If you do not have a “deep fry” thermometer, dip a wooden chopstick or wooden spoon handle into the oil. If the oil slowly starts to bubble and the bubbles are small, then the oil is hot enough for frying. If the oil bubbles rapidly, with large bubbles, then the oil is too hot.]*
 - ▶ Cook potato strips, such as French fries, to a golden yellow rather than a golden brown color. *[Longer cooking times result in greater formation of acrylamide.]*
 - ▶ Toast bread to the lightest color acceptable.
 - ▶ Soak raw potato slices in water for 15-30 minutes before frying or roasting. Drain and blot dry before cooking. *[Soaking in water removes some of the precursors to acrylamide formation.]*
- ❌ Do not store raw potatoes in the refrigerator. *[Cold temperatures increase the sugar content of potatoes. Sugars are precursors to acrylamide formation.]*

For more information:

General Acrylamide Fact Sheets and Resources:

- American Cancer Society
 - ▶ Acrylamide and Cancer Risk
<http://www.cancer.org/cancer/cancercauses/othercarcinogens/athome/acrylamide>

Acrylamide in Food:

- US Department of Health and Human Services (HHS)
 National Institute of Environmental Health Sciences (NIEHS)
 - ▶ Acrylamide
<https://www.niehs.nih.gov/health/topics/agents/acrylamide/index.cfm>
- National Institute of Health, National Cancer Institute (NIH-NCI)
 - ▶ Acrylamide in Food and Cancer Risk
<http://www.cancer.gov/about-cancer/causes-prevention/risk/diet/acrylamide-fact-sheet>
- The US Food and Drug Administration (FDA)
 - ▶ Acrylamide:
<http://www.fda.gov/Food/FoodborneIllnessContaminants/ChemicalContaminants/ucm2006782.htm>

OEHHA

Acrylamide

Proposition 65:

- California Environmental Protection Agency (CalEPA)
Office of Environmental Health Hazard Assessment (OEHHA)
 - ▶ Proposition 65: Background:
<https://www.p65warnings.ca.gov/faq>
 - ▶ Proposition 65: The Chemical List:
<https://www.p65warnings.ca.gov/chemicals>

Scientific Information on Acrylamide:

- California Environmental Protection Agency (CalEPA)
Office of Environmental Health Hazard Assessment (OEHHA)
 - ▶ Characterization of Acrylamide Intake from Certain Foods:
<http://oehha.ca.gov/media/downloads/cnr/acrylamideintakereport.pdf>
- National Toxicology Program (NTP)
 - ▶ NTP Brief on Acrylamide:
https://ntp.niehs.nih.gov/ntp/ohat/acrylamide/acrylamide_monograph.pdf

EXHIBIT 10

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K. McCoy, Exec. Off./Clerk

*Attorneys for People of the State of California
ex rel. Edmund G. Brown Jr., Attorney General of the
State of California*

**SUPERIOR COURT OF THE STATE OF CALIFORNIA
FOR THE COUNTY OF ALAMEDA**

PEOPLE OF THE STATE OF CALIFORNIA
ex rel. EDMUND G. BROWN JR.,
ATTORNEY GENERAL OF THE STATE OF
CALIFORNIA,

Plaintiff,

v.

SNYDER'S OF HANOVER, INC., BIRDS
EYE FOODS, INC., CORAZONAS FOOD,
INC., FRITO-LAY, INC., GRUMA
CORPORATION, H.J. HEINZ COMPANY,
L.P., KETTLE FOODS, INC., LANCE, INC.,
RESERVE BRANDS, INC., SNAK KING
CORPORATION, and DOES 1 through 100,

Defendants.

CASE NO.: RG 09455286

ASSIGNED FOR ALL PURPOSES TO:

JUDGE: Hon. Steven A. Brick

DEPT: 17

**[PROPOSED] AMENDED CONSENT
JUDGMENT AS TO DEFENDANT FRITO-
LAY, INC.**

Date: September 19, 2011

Time: 3:00 p.m.

Dept: 17

Judge: Honorable Steven A. Brick

Reservation No.: R-1210296

Trial Date: None set.

Action Filed: June 1, 2009

1 **1. INTRODUCTION**

2 1.1. On June 1, 2009, the People of the State of California, *ex rel.* the Attorney General
3 of the State of California (the “People” or the “Attorney General”), filed a complaint for civil
4 penalties and injunctive relief for violations of Proposition 65 and unlawful business practices in the
5 Superior Court for the County of Alameda. The People’s Complaint alleges that the Defendants
6 failed to provide clear and reasonable warnings that ingestion of the products identified in the
7 Complaint would result in exposure to acrylamide, a chemical known to the State of California to
8 cause cancer. The Complaint further alleges that under the Safe Drinking Water and Toxic
9 Enforcement Act of 1986, Health and Safety Code section 25249.6 *et seq.*, also known as
10 “Proposition 65,” businesses must provide persons with a “clear and reasonable warning” before
11 exposing individuals to these chemicals, and that the Defendants failed to do so. The Complaint
12 also alleges that these acts constitute unlawful acts in violation of the Unfair Competition Law,
13 pursuant to Business and Professions Code sections 17200 *et seq.*

14 1.2. Frito-Lay, Inc. (“Settling Defendant”) is among the Defendants named in the
15 Complaint. Both the People and Settling Defendant shall be referred to as a “Party” to this Consent
16 Judgment, and collectively they shall be referred to herein as the “Parties” to this Consent Judgment.

17 1.3. Settling Defendant is a Delaware corporation that employs more than ten employees,
18 and has employed more than ten employees at some time relevant to the allegations of the
19 complaint, and that manufactures, distributes and/or sells products in the State of California and has
20 done so in the past.

21 1.4. The products covered by this Consent Judgment (hereinafter, “Covered Products”)
22 are those snack food products manufactured and sold by Settling Defendant or its Affiliates (as
23 defined in Paragraph 8.1 herein) that are identified in Exhibit A, including corn chips, corn puffs,
24 pork rinds, bagel chips, pita chips, pretzels, tortilla chips, multigrain chips, vegetable chips, and
25 popcorn, and excluding Potato Crisp Products and Potato Chip Products covered by the prior
26 Consent Judgment as to Defendant Frito-Lay, Inc. in *People v. Frito-Lay, Inc., et al.*, Case No. BC
27 338956, Los Angeles Superior Court (Aug. 1, 2008). After the Effective Date, should Settling
28 Defendant introduce for sale to consumers in California a snack food product not described in

1 Exhibit A, then Settling Defendant shall give notice of such to the Attorney General in the form of a
2 revised version of Exhibit A. Should the Attorney General object to such notice within 30 days
3 following receipt of such notice, then the Parties shall proceed in accordance with Paragraph 5.1;
4 otherwise, this Consent Judgment shall be deemed to be modified to include such product as a
5 Covered Product.

6 1.5. For purposes of this Consent Judgment only, the People and the Settling Defendant
7 stipulate that this Court has jurisdiction over the allegations of violations contained in the People's
8 Complaint and personal jurisdiction over Settling Defendant as to the acts alleged in the People's
9 Complaint, that venue is proper in the County of Alameda, and that this Court has jurisdiction to
10 enter this Consent Judgment as a full and final resolution of all claims which were or could have
11 been raised in the Complaint based on the facts alleged therein.

12 1.6. The People and Settling Defendant stipulate to the entry of this Consent Judgment as
13 a full and final settlement of all claims that were raised in the Complaint (except as specified in
14 Paragraph 8.1 herein), arising out of the facts or conduct alleged therein. Except as expressly set
15 forth herein, nothing in this Consent Judgment shall prejudice, waive or impair any right, remedy,
16 or defense the Attorney General and Settling Defendant may have in any other or in future legal
17 proceedings unrelated to these proceedings. However, this paragraph shall not diminish or
18 otherwise affect the obligations, responsibilities, and duties of the Parties under this Consent
19 Judgment.

20 1.7. By stipulating to the entry of this Consent Judgment and agreeing to provide the
21 relief and remedies specified herein, Settling Defendant does not admit (a) that it has violated, or
22 threatened to violate Proposition 65 or Business and Professions Code sections 17200 *et seq.*, or any
23 other law or legal duty; or (b) that the chemical acrylamide in food poses any risk to human health.
24 The Parties recognize that acrylamide is naturally formed when certain foods, such as the snack
25 food products at issue in this case, are heated, and that levels of acrylamide formation are due to a
26 wide variety of factors in the raw material and that may vary from location to location. Settling
27 Defendant contends that the Target Level set in this Consent Judgment is based on specific factors
28

1 that affect acrylamide levels in the Covered Products manufactured in or near California, and that
2 the Target Level is not relevant in areas outside of California where these same factors vary.

3 1.8. The Effective Date of this Amended Consent Judgment shall be the date on which
4 the original Consent Judgment was entered as a judgment by the Superior Court, that is, April 28,
5 2010.

6 **2. INJUNCTIVE RELIEF: ACRYLAMIDE REDUCTION**

7 2.1. *Target Level and Target Date.* Settling Defendant shall reduce the level of
8 acrylamide in its Covered Products shipped for sale in California after September 30, 2011 (the
9 “Target Date”) to a level of 281 parts per billion, measured by the weighted arithmetic mean
10 pursuant to the protocol described in Paragraph 2.3 (the “Target Level”) or be subject to the
11 provisions of Paragraph 3. Settling Defendant shall continue its program of research, development,
12 and implementation of technologies and methods intended to reduce the presence of acrylamide in
13 the Covered Products shipped for sale in California. Settling Defendant shall endeavor in good
14 faith, using commercially and technologically reasonable efforts, to achieve the Target Level in the
15 Covered Products shipped for sale in California by the Target Date. In addition, for the purposes of
16 this Consent Judgment, Settling Defendant shall not be considered to have achieved the Target
17 Level if, as of the Target Date, the arithmetic mean of the acrylamide concentration in any Group of
18 Covered Products, as set forth in Exhibit A and as determined in accordance with the protocol
19 described in Paragraph 2.3, exceeds the Target Level by more than 25%.

20 2.1.1: Notwithstanding any other provision of this Consent Judgment, on or before
21 the Target Date, Settling Defendant shall submit to the Attorney General a report demonstrating that
22 it has achieved the Target Level, as defined in Section 2.1, without including the Covered Products
23 in Group C. If Settling Defendant achieves the Target Level (without including the Covered
24 Products in Group C) by September 30, 2011, then, notwithstanding any other provision of this
25 Consent Judgment, the Target Date shall be extended to December 31, 2011.

26 2.2. “Shipped for sale in California” means Covered Products that Settling Defendant
27 either directly ships into California for sale in California or that it sells to a distributor who Settling
28 Defendant knows will sell the Covered Products to consumers in California. Where a retailer or

1 distributor sells Covered Products both in California and other states, Settling Defendant shall take
2 commercially reasonable steps to ensure that, after the Target Level has been reached, the only
3 Covered Products that are sold in California are either (i) Covered Products included in the
4 weighted arithmetic mean for which the Target Level has been achieved; or (ii) Covered Products
5 for which Settling Defendant has complied with Paragraph 3.

6 2.3. *Testing.*

7 (a) Testing for acrylamide shall be performed using either GC/MS (Gas
8 Chromatography/Mass Spectrometry), LC-MS/MS (Liquid Chromatograph-Mass
9 Spectrometry/Mass Spectrometry), or any other testing method agreed upon by the Parties to this
10 Consent Judgment.

11 (b) Representative samples of each of the Covered Products to be tested for purposes of
12 demonstrating compliance with the Target Level must be taken over no less than a ten-day period
13 from at least ten batches of such Covered Products produced at locations that supply such Covered
14 Products to California.

15 (c) To comply with the Target Level, testing must establish that the weighted arithmetic
16 mean of the samples is at or below the Target Level with a 95% confidence level, i.e., $p < 0.05$, using
17 stratified random sampling.

18 (d) The weighted arithmetic mean is to be calculated by the following formula: Multiply
19 the arithmetic mean of the acrylamide concentration (established by the sampling methodology) of
20 all products within a Group (as set forth in Exhibit A) by that Group's fraction of total sales volume
21 (net of returns) for all Groups to be included in the weighted arithmetic mean of the Covered
22 Products, and thereafter sum all such adjusted concentrations for all Groups that are required to be
23 included in the weighted arithmetic mean. Sales volume for each Group and for total sales volume
24 for the Covered Products shall be based upon the most current 52 week IRI InfoScan data (in dollars,
25 net of returns) for the Los Angeles, San Francisco/Oakland, San Diego and Sacramento
26 metropolitan areas available to Settling Defendant as of the date of sampling.
27
28

1 (e) All test results of acrylamide concentrations, once provided to the Attorney General,
2 shall be public documents, but nothing in this Consent Judgment shall preclude Settling Defendant
3 from claiming business confidentiality as to sales volumes of any or all of the Covered Products.

4 (f) Testing of Covered Products to demonstrate compliance with this Paragraph 2 shall
5 be conducted and/or supervised by either (i) a third party under contract to and paid by Settling
6 Defendant or (ii) with the Attorney General's prior approval, Settling Defendant itself under a
7 protocol previously approved by the Attorney General.

8 2.4. *Verification and Warnings*

9 (a) If Settling Defendant's test results demonstrate that the Target Level has been
10 achieved for the Covered Products, Settling Defendant shall be required to test each of the Covered
11 Products on two additional occasions only: once during the first year and once during the second
12 year after the, Target Level has been achieved, provided that there is at least a six-month interval
13 between these two testing occasions. If those tests confirm that the Target Level has been achieved
14 for the Covered Products, Settling Defendant shall have no further duty to test the Covered Products.

15 (b) If Settling Defendant has not achieved the Target Level for the Covered Products by
16 the Target Date (including any extensions provided under Paragraph 2.5), it shall provide warnings
17 for the Covered Products as provided herein in Paragraph 3. Settling Defendant may also continue
18 testing of the Covered Products until tests demonstrate that the Target Level has been achieved for
19 the Covered Products, at which time Settling Defendant shall have no further duty to warn.

20 (c) After Settling Defendant has demonstrated that the Target Level has been achieved
21 and has fulfilled its duty to test the Covered Products, if the Attorney General believes that the
22 Target Level has not been achieved, he may apply to the Court for enforcement of this Consent
23 Judgment. Any test data used by the Attorney General for this purpose must be performed and
24 analyzed by methods consistent with Paragraph 2.3(a) and include at least ten samples of each
25 Group of the Covered Products. A prima facie showing of violation based on such test results may
26 be rebutted by a showing made in compliance with all aspects of the testing and sampling protocol
27 of Paragraph 2.3.
28

1 2.5. *Extension of Target Dates.* At least 90 days prior to the Target Date, Settling
2 Defendant may initiate a meet and confer session with the Attorney General regarding a possible
3 extension of the Target Date. Upon timely application to the Court prior to the passing of the
4 Target Date, and for good cause shown based on Settling Defendant's diligence and good faith
5 efforts as well as reported progress to date, this Consent Judgment shall be modified to extend the
6 Target Date by no more than three (3) months.

7 2.6. *Technology Licensing.* The requirements in this Consent Judgment are not
8 contingent upon the use of any particular method to achieve the Target Level, but Settling
9 Defendant shall license any patented technology used to meet the Target Level, whether existing or
10 in the future, to others for use in other food products, at a commercially reasonable price, and using
11 other commercially reasonable terms.

12 **3. INJUNCTIVE RELIEF: CLEAR AND REASONABLE WARNINGS**

13 3.1. *Warnings in General.* If Settling Defendant does not achieve the Target Level by the
14 applicable Target Date, Settling Defendant shall within 30 days and until such time as it achieves
15 the Target Level provide warnings either:

16 (a) by placing a warning label as described in Paragraph 3.2 on the package of all
17 Covered Products that Settling Defendant would be required to exclude from the calculation of the
18 weighted arithmetic mean to achieve the Target Level for the Covered Products; or, at Settling
19 Defendant's option,

20 (b) by providing signs as described in Paragraph 3.3 for all Covered Products that
21 Settling Defendant would be required to exclude from the calculation of the weighted arithmetic
22 mean to achieve the Target Level for the Covered Products.

23 3.2. *Label Warnings.* A label warning placed on the package of a Covered Product
24 pursuant to Paragraph 3.1(a) shall either (a) conform to the requirements for the "safe harbor"
25 warning methods set out in Cal. Code Regs., tit. 27, § 25601, and, at the Settling Defendant's option,
26 may also state that acrylamide is the chemical in question and/or the approximate level of
27 acrylamide in the product; or (b) provide substantially the same information as set forth for sign
28 warnings in Paragraph 3.3(b).

3.3. Sign Warnings.

(a) *Form of Sign.* A warning sign shall be rectangular and at least 36 square inches in size, with the word “WARNING” centered one-half of an inch from the top of the sign in ITC Garamond bold condensed type face all in one-half inch capital letters. The body of the warning message shall be in ITC Garamond bold condensed type face. For the body of the warning message, left and right margins of at least one-half of an inch, and a bottom margin of at least one-half inch shall be observed. Larger signs shall bear substantially the same proportions of type size and spacing to sign dimension as a sign that is 36 square inches in size.

(b) *Text of Sign.* Unless modified by agreement of the Parties to this Consent Judgment, the sign shall contain the following text:

WARNING

Certain potato- and/or grain-based snack food products, such as [list specific products as applicable] contain acrylamide, a substance identified as causing cancer under California's Proposition 65. [At Settling Defendant's option, the following sentence may also be added: Certain other cooked foods that have been roasted or browned, such as french fries, crackers, cookies, coffee, breads, and cereals, also contain acrylamide, although in varying amounts.]

Acrylamide is not added to these foods but is created when these and certain other foods are browned.

The FDA has not advised people to stop eating these snack food products or any other foods containing acrylamide as a result of cooking. For more information, see www.fda.gov.

(c) *Placement of Sign.* The sign shall be posted on the shelf(ves) or in the aisle(s) where the Covered Products for which the warning is being provided are sold; unless the store has less than 7,500 square feet of retail space and no more than two cash registers, in which case it may be placed at each cash register. Should Settling Defendant, in conjunction with one or more retailers, desire to provide the warning via sales receipts or other information provided to each customer at checkout, or should Proposition 65 or its implementing regulations be changed from their terms as they exist on the date of entry of this Consent Judgment to provide a new manner or language for an optional safe harbor warning, then Settling Defendant shall meet and confer with the Attorney

1 General and, following agreement, jointly apply to the Court for approval of a plan for
2 implementing warnings in such manner. Such plan shall be approved only upon a showing that the
3 warning provided in such manner will comply with the law and be at least as effective as the forms
4 of warnings otherwise required by this Consent Judgment.

5 (d) *Distribution.* Settling Defendant (or its agent) shall provide signs to retailers who
6 operate retail locations in California that are collectively responsible for at least 70 percent of
7 Settling Defendant's sales in the State of California of Covered Products for which the warning is
8 being provided. Signs shall be provided with a letter substantially as provided in Exhibit B, in
9 which posting instructions are provided. The letter shall request that the receiving retailer provide
10 Settling Defendant a written acknowledgment that the sign will be posted. Settling Defendant shall
11 send a follow up letter substantially as provided in Exhibit C to the same retailers who were sent the
12 original letter and who did not send any acknowledgment. Settling Defendant (or its agent) shall
13 maintain files demonstrating compliance with this provision, including the letters sent and receipts
14 of any acknowledgments from retailers, which shall be provided to the Attorney General on written
15 request.

16 3.4. *Option to Provide Warnings.*

17 (a) With respect to the Covered Products, Settling Defendant may opt to provide
18 warnings under Paragraph 3.1 and cease its acrylamide reduction efforts under Paragraph 2 if either
19 or both of the following conditions have been satisfied with respect to the Covered Products: (i)
20 acrylamide warnings covering one or more products manufactured and sold by other companies that
21 are of the same type as the Covered Products appear on packages of such products accounting for
22 20% of sales of all such products in California that are not produced by Settling Defendant, based
23 on IRI sales data; and/or (ii) non-package acrylamide warnings specifically mentioning one or more
24 such products appear at 500 or more store locations in California.

25 (b) If Settling Defendant believes either or both conditions has/have occurred with
26 respect to the Covered Products, it shall give notice of such to the Attorney General, together with
27 documentation evidencing such occurrence. Following such notice, Settling Defendant and the
28 Attorney General will promptly meet and confer regarding the situation, and following that meet

and confer period of no longer than 30 days, Settling Defendant, by giving further notice of at least 30 days to the Attorney General, which the Attorney General may extend, at his option, by up to 60 days, may elect to (i) cease acrylamide reduction efforts with respect to the Covered Products; (ii) provide the warnings required by Paragraph 3.1 for the Covered Products; and (iii) within 30 days make all remaining payments required by Paragraph 4 with respect to the Covered Products.

3.5. *Extra-Territorial Effect.* Nothing in this Consent Judgment requires that warnings be given for any Covered Products sold outside the State of California.

3.6. *Cessation of Warnings.* If Settling Defendant has demonstrated by testing that it has achieved the Target Level for any or all Covered Products after providing warnings for such Covered Products under Paragraph 3, then Settling Defendant may cease providing warnings for such Covered Products.

4. PAYMENTS

4.1. *Initial Civil Penalty.* Settling Defendant shall pay a civil penalty to the Attorney General pursuant to Health & Safety Code section 25249.12 of \$375,000 no later than 30 days after the Effective Date.

4.2. *Interim Civil Penalty.* As an incentive for early achievement in acrylamide reduction, Settling Defendant shall pay an additional civil penalty to the Attorney General pursuant to Health & Safety Code section 25249.12 of \$550,000 ("Interim Civil Penalty") no later than six months after the Effective Date, but if Settling Defendant has achieved the Target Level for one or more of the Groups specified in Exhibit A before such Interim Civil Penalty is due, then a portion of the Interim Civil Penalty will be waived in proportion to the percentage of total sales volume of the Covered Products represented by the sales volume of the Group or Groups for which Settling Defendant has achieved the Target Level (the Group's "pro rata share"), so that if Settling Defendant has achieved the Target Level (as defined in Paragraph 2.1) with respect to all Covered Products before such payment is due, the entire Interim Civil Penalty shall be waived. Each Group's pro rata share of the Interim Civil Penalty is to be calculated by the following formula: Multiply that Group's fraction of the total sales volume (net of returns) for all Groups listed in Exhibit A by \$550,000. Sales volume for each Group and for total sales volume for the Covered

1 Products shall be based upon the most current 52 week IRI InfoScan data (in dollars, net of returns)
2 for the Los Angeles, San Francisco/Oakland, San Diego and Sacramento metropolitan areas
3 available to Settling Defendant as of 30 days before the date the Interim Civil Penalty is due.

4 4.3. *Final Civil Penalties.* As a further incentive for early achievement in acrylamide
5 reduction, Settling Defendant shall pay an additional civil penalty ("Final Civil Penalty") to the
6 Attorney General pursuant to Health & Safety Code section 25249.12 of \$1,700,000 no later than
7 the Target Date (without considering any extensions provided under Paragraph 2.5), but if Settling
8 Defendant has achieved the Target Level before the Target Date (without considering any
9 extensions provided under Paragraph 2.5), such Final Civil Penalty shall be waived.

10 4.4. *Enforcement Fund Payment.* Within 30 days of the Effective Date, Settling
11 Defendant shall pay \$50,000 to be used by the Attorney General for the enforcement of Proposition
12 65. Funds paid pursuant to this paragraph shall be placed in an interest-bearing Special Deposit
13 Fund established by the Attorney General. These funds, including any interest, shall be used by the
14 Attorney General, until all funds are exhausted, for the costs and expenses associated with the
15 enforcement and implementation of Proposition 65, including investigations, enforcement actions,
16 other litigation or activities as determined by the Attorney General to be reasonably necessary to
17 carry out his duties and authority under Proposition 65. Such funding may be used for the costs of
18 the Attorney General's investigation, filing fees and other court costs, payment to expert witnesses
19 and technical consultants, purchase of equipment, travel, purchase of written materials, laboratory
20 testing, sample collection, or any other cost associated with the Attorney General's duties or
21 authority under Proposition 65. Funding placed in the Special Deposit Fund pursuant to this
22 paragraph, and any interest derived therefrom, shall solely and exclusively augment the budget of
23 the Attorney General's Office and in no manner shall supplant or cause any reduction of any portion
24 of the Attorney General's budget.

25 4.5. *Delivery.* Each payment required by this Consent Judgment shall be made through
26 the delivery of separate checks payable to "California Department of Justice," to the attention of
27 Laura J. Zuckerman, Deputy Attorney General, California Department of Justice, 1515 Clay Street,
28 20th Floor, Oakland, CA 94612, with a copy of the check and cover letter to be sent to Robert

1 Thomas, Legal Analyst, California Department of Justice, 1515 Clay Street, 20th Floor, Oakland,
2 CA 94612.

3 **5. MODIFICATION OF CONSENT JUDGMENT**

4 5.1. *Procedure for Modification.* Except as provided in Paragraph 1.4, this Consent
5 Judgment may be modified by written agreement of the Attorney General and Settling Defendant,
6 after noticed motion, and upon entry of a modified consent judgment by the Court thereon, or upon
7 motion of the Attorney General or Settling Defendant as provided herein or as otherwise provided
8 by law, and upon entry of a modified consent judgment by the Court. Before filing an application
9 with the Court for a modification to this Consent Judgment, Settling Defendant shall meet and
10 confer with the Attorney General to determine whether the Attorney General will consent to the
11 proposed modification. If a proposed modification is agreed upon, then Settling Defendant and the
12 Attorney General will present the modification to the Court by means of a stipulated modification to
13 the Consent Judgment. Otherwise, Settling Defendant shall bear the burden of establishing that the
14 modification is appropriate based on the occurrence of a condition set forth in this Consent
15 Judgment or as otherwise provided by law.

16 5.2. *Duty to Warn.* If the Attorney General agrees in a settlement or judicially entered
17 consent judgment that one or more products manufactured and sold by other companies that are of
18 the same type as the Covered Products do not require a warning for acrylamide under Proposition
19 65, or if a court of competent jurisdiction renders a final judgment, and the judgment becomes final,
20 that one or more products manufactured and sold by other companies that are of the same type as
21 the Covered Products do not require a warning for acrylamide under Proposition 65, then the duty to
22 warn under Paragraph 3 of this Consent Judgment and the duty to reduce acrylamide levels under
23 Paragraph 2 of this Consent Judgment shall be eliminated with respect to such portion (or all) of the
24 Covered Products as is appropriate, except that, in the event that such final judgment is not binding
25 on the Attorney General, the Court may determine whether (or the extent to which) Settling
26 Defendant's duties should be eliminated or modified considering other equitable and legal factors.

27 5.3. *Manner or Form of Warning.* If the Attorney General subsequently agrees in a
28 settlement or judicially entered consent judgment, or if a court of competent jurisdiction renders a

1 final judgment, and the judgment becomes final, that warnings under Proposition 65 (based on the
2 presence of acrylamide) for one or more products manufactured and sold by other companies that
3 are of the same type as the Covered Products may be provided in a manner or form different from
4 that set forth in this Consent Judgment, then the manner and form of warning set forth in this
5 Consent Judgment shall be modified to entitle Settling Defendant to provide warnings in such other
6 manner or form, except that, in the event that such final judgment is not binding on the Attorney
7 General, the Court may determine whether (or the extent to which) Settling Defendant's duties
8 should be eliminated or modified considering other equitable and legal factors.

9 5.4. *Change in Proposition 65.* If Proposition 65 or its implementing regulations
10 (including the "safe harbor no significant risk level" for acrylamide set forth at Cal. Code Regs., tit.
11 27, section 25705, subdivision (c)(2)) are changed from their terms as they exist on the date of entry
12 of this Consent Judgment to establish that warnings for acrylamide in some or all of the Covered
13 Products are not required, then this Consent Judgment will be modified to relieve Settling
14 Defendant of its obligations with respect to such portion of the Covered Products as is appropriate.
15 The Parties recognize that the Target Level is based on a compromise of a number of issues, and
16 that an increase in the "safe harbor no significant risk level" above the current 0.2 micrograms per
17 day would not necessarily entitle Settling Defendant to a modification of the terms of this Consent
18 Judgment.

19 5.5. *Federal Preemption.* If a court of competent jurisdiction or an agency of the federal
20 government, including, but not limited to the U.S. Food and Drug Administration, states through
21 any regulation or legally binding act that federal law has preemptive effect on any of the
22 requirements of this Consent Judgment, including, but not limited to precluding Settling Defendant
23 from providing any of the warnings set forth in this Consent Judgment or the manner in which such
24 warning are given, then this Consent Judgment will be modified to bring it into compliance with or
25 avoid conflict with federal law, but the modification shall not be granted unless this Court
26 concludes, in a final judgment or order, that such modification is necessary to bring this Consent
27 Judgment into compliance with or avoid conflict with federal law. Specifically, a determination
28 that the provision of some, but not all, forms of warning described in Paragraph 3 above is not

1 permitted shall not relieve Settling Defendant of the duty to provide one of the other warnings
2 described under this judgment for which such determination has not been made.

3 5.6. *Scientific Review.* If an agency of the federal government, including but not limited
4 to the U.S. Food-and Drug Administration, determines in an official communication, regulation, or
5 legally binding act, following a thorough review of the available scientific studies and opportunity
6 for public comment, a cancer potency estimate (Q*) for acrylamide that equates to a no significant
7 risk level of 1.0 meg/day or higher, Settling Defendant or its representative (including a coalition or
8 trade association) may petition the California Office of Environmental Health Hazard Assessment
9 (“OEHHHA”) to revise the no significant risk level for acrylamide set forth at Cal. Code Regs., tit. 27,
10 section 25705, subdivision (c)(2), in light of such federal action. If the Target Date (including any
11 extensions under Paragraph 2.5) falls after the date of the federal agency determination noted above,
12 but before OEHHHA has issued a final decision on the petition, then the Target Date will be extended
13 to such date as is 90 days after the date on which OEHHHA issues a final decision on such petition.

14 **6. ENFORCEMENT**

15 6.1. The People may, by motion or application for an order to show cause before this
16 Court, enforce the terms and conditions contained in this Consent Judgment. In any such
17 proceeding, the People may seek whatever fines, costs, penalties, or remedies are provided by law
18 for failure to comply with the Consent Judgment and where said violations of this Consent
19 Judgment constitute subsequent violations of Proposition 65 or other laws independent of the
20 Consent Judgment and/or those alleged in the Complaint, the People are not limited to enforcement
21 of the Consent Judgment, but may seek in another action whatever fines, costs, penalties, or
22 remedies are provided for by law for failure to comply with Proposition 65 or other laws. In any
23 action brought by the People alleging subsequent violations of Proposition 65 or other laws, Settling
24 Defendant may assert any and all defenses that are available.

25 **7. AUTHORITY TO STIPULATE TO CONSENT JUDGMENT**

26 7.1. Each signatory to the Parties’ stipulation for entry of this Consent Judgment certifies
27 that he or she is fully authorized by the Party he or she represents to stipulate to this Consent
28

1 Judgment and to enter into and execute the stipulation on behalf of the Party represented and legally
2 to bind that Party.

3 **8. CLAIMS COVERED**

4 8.1. This Consent Judgment is a full, final, and binding resolution between the People
5 and Settling Defendant of any violation of Proposition 65, Business & Professions Code sections
6 17200 *et seq.*, or any other statutory or common law claims that have been or could have been
7 asserted in the Complaint against Settling Defendant for failure to provide clear and reasonable
8 warnings of exposure to acrylamide from the consumption of the Covered Products, or any other
9 claim based on the facts or conduct alleged in the Complaint as to the Covered Products, whether
10 based on actions committed by Settling Defendant or by any entity to whom it distributes or sells
11 Covered Products, or any entity that sells the Covered Products to consumers in the state of
12 California except for sales of Covered Products by retailers during any period in which such
13 retailers have not posted signs sent to them pursuant to Paragraph 3.3(d). With this one exception,
14 as to Covered Products, compliance with the terms of this Consent Judgment resolves any issue
15 now, in the past, and in the future concerning compliance by Settling Defendant, its parents,
16 shareholders, divisions, subdivisions, subsidiaries, sister companies, affiliates, franchisees,
17 cooperative members, and licensees; their distributors, wholesalers, and retailers who sell Covered
18 Products; and the predecessors, successors, and assigns of any of them (collectively, "Affiliates"),
19 with the requirements of Proposition 65 as to acrylamide in the Covered Products.

20 **9. RETENTION OF JURISDICTION**

21 9.1. This Court shall retain jurisdiction of this matter to implement the Consent Judgment.

22 **10. PROVISION OF NOTICE**

23 10.1. When any Party is entitled to receive any notice under this Consent Judgment, the
24 notice shall be sent by overnight courier service to the person and address set forth in this Paragraph.
25 Any Party may modify the person and address to whom the notice is to be sent by sending the other
26 Party notice by certified mail, return receipt requested. Said change shall take effect for any notice
27 mailed at least five days after the date the return receipt is signed by the Party receiving the change.

28 10.2. Notices shall be sent to:

For the People/the Attorney General:

Laura J. Zuckerman
Timothy E. Sullivan
Deputy Attorneys General
1515 Clay Street, 20th Floor
Oakland, CA 94612

For Frito-Lay, Inc.:

Attn: General Counsel
Frito-Lay, Inc.
7701 Legacy Drive
Plano, TX 75024-4099

with a copy to:

Trenton H. Norris
1 Embarcadero Center, Floor 22

San Francisco, CA 94111

11. COURT APPROVAL

11.1. This Amended Consent Judgment shall be submitted to the Court pursuant to the Court's September 15, 2011 tentative order granting the [Proposed] Order for Modification to Frito-Lay Consent Judgment.

12. ENTIRE AGREEMENT

12.1. This Consent Judgment contains the sole and entire agreement and understanding of the Parties with respect to the entire subject matter hereof, and any and all prior discussions, negotiations, commitments and understandings related hereto. No representations, oral or otherwise, express or implied, other than those contained herein have been made by any Party hereto. No other agreements not specifically referred to herein, oral or otherwise, shall be deemed to exist or to bind any of the Parties.

IT IS SO ORDERED, ADJUDGED, AND DECREED:

Dated:

SEP 19 2011

STEVEN A. BRICK

Hon. Steven A. Brick
Judge of the Superior Court

Exhibit A

COVERED PRODUCTS

CORN/TORTILLA CHIPS

GROUP A. Fritos, Doritos, Tostitos, Santitas (all flavors, excluding Baked)

PRETZELS

GROUP B. Rold Gold (all flavors)

POPCORN

GROUP C. Cracker Jack

GROUP D. SmartFood, Chester's (all flavors)

PUFF EXTRUDED CORN

GROUP E. Cheetos, Chester's Puffcorn (all flavors, excluding Baked)

GROUP F. Chester's Fries

ALL OTHER NON-POTATO

GROUP G. Flat Earth, Sabritones, Munchies

GROUP H. Sunchips, Stacy's Soy, Funyuns, Stacy's Pita, Stacy's Bagel, Baken-Ets, Maui Style Shrimp Chips

BAKED

GROUP I. All of the above products sold under the subbrand "Baked":
Baked Doritos, Baked Tostitos, Baked Cheetos

Exhibit B

(For use if Settling Defendant provides sign warnings pursuant to Paragraph 3.3)

**THIS COMMUNICATION APPLIES ONLY TO
RETAIL LOCATIONS IN CALIFORNIA**

Frito-Lay, Inc. has entered into a consent judgment with the Attorney General for the State of California regarding the presence of acrylamide in specified snack food products sold by retailers at retail locations in California.

Under the terms of this consent judgment, Frito-Lay, Inc. is providing the enclosed sign warnings to retailers to be posted in retail stores selling any of the specified snack food products identified below in California. In the consent judgment, Frito-Lay, Inc. obtained a conditional release on your behalf. For the release to continue to be effective after the date of this letter, you need to comply with the directions in this communication.

We request that you post these signs on your shelf(ves) or in your aisle(s) where the identified products are sold. For stores with less than 7,500 square feet of retail space and no more than two cash registers, the sign may be placed at each cash register instead of on the shelf(ves) or in the aisle(s).

Please sign and return the written acknowledgement below to acknowledge that you have received the signs and that they will be posted in accordance with these specifications until you receive written instruction from Frito-Lay, Inc. to the contrary.

Thank you for your cooperation. If you need more signs or have any questions, such as the appropriate sign locations for your specific retail store(s), please contact _____.

Acknowledged by:

(Signature)
(Print Name)
(Company/Store Location)
(Date)

List of Products

Exhibit C

(For use if Settling Defendant provides sign warnings pursuant to Paragraph 3.3)

**THIS COMMUNICATION APPLIES ONLY TO
RETAIL LOCATIONS IN CALIFORNIA**

On [Date], Frito-Lay, Inc. sent you a letter enclosing sign warnings for posting in your store(s) in California pursuant to a consent judgment entered into between Frito-Lay, Inc. and the Attorney General for the State of California regarding the presence of acrylamide in specified snack food products sold by retailers at retail locations in California.

These signs are to be posted on your shelf(ves) or in your aisle(s) where any of the specified snack food products identified below are sold in your stores in California. For stores with less than 7,500 square feet of retail space and no more than two cash registers, the sign may be placed at each cash register instead of on the shelf(ves) or in the aisle(s).

As stated in our prior letter, Frito-Lay, Inc. obtained a conditional release in the consent judgment on your behalf. For the release to be effective after the date of the prior letter, you need to comply with the directions in this communication.

We have not received your written acknowledgement that you have received the signs and that your store(s) will post these signs. Please sign and return the written acknowledgement below to acknowledge that you have received the signs and that they will be posted in accordance with these specifications until you receive written instruction from Frito-Lay, Inc. to the contrary.

Thank you for your cooperation. If you need more signs or have any questions, such as the appropriate sign locations for your specific retail store(s), please contact _____.

Acknowledged by:

(Signature)
(Print Name)
(Company/Store Location)
(Date)

List of Products

EXHIBIT 11

ENDORSED
FILED
ALAMEDA COUNTY

OFC 18 2018

CLERK OF THE SUPERIOR COURT
By: ANGEL LOGAN
DEPUTY

SUPERIOR COURT OF THE STATE OF CALIFORNIA
FOR THE COUNTY OF ALAMEDA

CENTER FOR ENVIRONMENTAL HEALTH,

Plaintiff,

v.

SNIKIDDY, LLC, *et al.*,

Defendants.

Case No. RG 16-838609

~~PROPOSED~~ CONSENT
JUDGMENT AS TO INVENTURE
FOODS, INC.

1. DEFINITIONS

1.1 The "Complaint" means the operative complaint in the above-captioned matter.

1.2 "Covered Products" means (1) all sweet potato-based snack food products manufactured by Settling Defendant; and (2) all Nathan's® brand Crunchy Crinkle Fries products manufactured by Settling Defendant. Without limitation, expressly excluded from "Covered Products" are: (a) all sliced potato products manufactured by Settling Defendant; (b) all potato-based "skin" products manufactured by Settling Defendant; and (c) all products covered by the Consent Judgment between the Environmental Law Foundation and Settling Defendant's predecessor Poore Brothers, Inc. in prior Proposition 65 litigation relating to acrylamide,

1 *Environmental Law Foundation v. Birds Eye Foods, Inc.*, Case No. BC356591, including those
 2 products identified in Section 1.7 thereof. A list of the Covered Products currently offered for
 3 sale by Settling Defendant is attached as Exhibit 1 hereto.¹

4 1.3 “Effective Date” means the date on which notice of entry of this Consent
 5 Judgment by the Court is served upon Settling Defendant.

6 **2. INTRODUCTION**

7 2.1 The Parties to this Consent Judgment are the Center for Environmental Health, a
 8 California non-profit corporation (“CEH”) and Inventure Foods, Inc. (“Settling Defendant”).
 9 CEH and Settling Defendant (the “Parties”) enter into this Consent Judgment to settle certain
 10 claims asserted by CEH against Settling Defendant as set forth, or could have been set forth, in
 11 the Complaint.

12 2.2 On or about August 12 and August 26, 2016, CEH provided 60-day Notices of
 13 Violation of Proposition 65 (the “Notices”) to the California Attorney General, the District
 14 Attorneys of every county in California, the City Attorneys of every California city with a
 15 population greater than 750,000, and to Settling Defendant, alleging that Settling Defendant,
 16 along with its downstream retailer and customer, Bristol Farms, violated Proposition 65 by
 17 exposing persons in California to acrylamide contained in Covered Products without first
 18 providing a clear and reasonable Proposition 65 warning.

19 2.3 Settling Defendant is a corporation or other business entity that manufactures,
 20 distributes, sells, and/or offers for sale Covered Products that are sold in the State of California or
 21 has done so at times relevant to the Complaint.

22 2.4 On November 10, 2016, CEH filed the initial complaint in the above-captioned
 23 matter, naming Settling Defendant, as well as Bristol Farms, as an original defendant. On April
 24

25 ¹ It is the Parties’ intent that the Extruded Products referenced in this Consent Judgment are the kind of products
 26 falling within Type 4 in the “extruded, pellet, and baked products” category in the Consent Judgment as to Defendant
 27 Snak King Corporation, entered August 31, 2011, in *People v. Snyder’s of Hanover, et al.*, Alameda County Superior
 28 Court Case No. RG 09-455286. These products are referred to as “Group C, Type 4” products in Exhibit A to the
 Snak King Consent Judgment, which is attached hereto as Exhibit 2 and available on the Attorney General’s website
 at <https://oag.ca.gov/prop65/litigation>.

11, 2017, CEH filed the Complaint, which added additional defendants but did not amend CEH's allegations or claims against Settling Defendant.

2.5 For purposes of this Consent Judgment only, the Parties stipulate that this Court has jurisdiction over the allegations of violations contained in the Complaint and personal jurisdiction over Settling Defendant as to the acts alleged in the Complaint, that venue is proper in the County of Alameda, and that this Court has jurisdiction to enter and enforce this Consent Judgment as a full and final resolution of all claims which were or could have been raised in the Complaint based on the facts alleged therein and in the Notices with respect to Covered Products manufactured, distributed, and/or sold by Settling Defendant.

2.6 Nothing in this Consent Judgment is or shall be construed as an admission against interest by the Parties of any fact, conclusion of law, issue of law, or violation of law, nor shall compliance with the Consent Judgment constitute or be construed as an admission against interest by the Parties of any fact, conclusion of law, issue of law, or violation of law. Nothing in this Consent Judgment shall prejudice, waive or impair any right, remedy, argument, or defense the Parties may have in any other pending or future legal proceedings. This Consent Judgment is the product of negotiation and compromise and is accepted by the Parties solely for purposes of settling, compromising, and resolving issues disputed in this action.

3. INJUNCTIVE RELIEF

3.1 **Reformulation of Covered Products.** Upon the Effective Date, Settling Defendant shall not manufacture, ship, sell, or offer for sale Covered Products that will be sold or offered for sale in California that exceed the following acrylamide concentration levels (the "Reformulation Levels"), such concentration to be determined by use of a test performed by an accredited laboratory using either GC/MS (Gas Chromatograph/Mass Spectrometry), LC-MS/MS (Liquid Chromatograph-Mass Spectrometry), or any other testing method agreed upon by the Parties:

3.1.1 The average acrylamide concentration shall not exceed 350 ppb by weight. The Average Level is determined by randomly selecting and testing at least 1 sample each from 5

different lots of a particular type of Covered Product (or the maximum number of lots available for testing if less than 5) during a testing period of at least 60 days. The mean and standard deviation shall be calculated using the sampling data. Any data points that are more than three standard deviations outside the mean shall be discarded once, and the mean and standard deviation recalculated using the remaining data points. The mean determined in accordance with the procedure shall be deemed the “Average Level.”

3.1.2 The acrylamide concentration of any individual unit of Covered Products shall not exceed 490 ppb by weight, based on a representative composite sample taken from the individual unit being tested (the “Unit Level”).

For avoidance of doubt, Covered Products either manufactured, shipped, or sold by Settling Defendant prior to the Effective Date are not subject to the Reformulation Levels, even if such products are sold in California or to California consumers after the Effective Date.

3.2 Clear and Reasonable Warnings. With the exception of the Covered Products identified on Exhibit A, a Covered Product purchased, manufactured, shipped, sold or offered for sale by Settling Defendant may, as an alternative to meeting the reformulation levels set forth in Section 3.1, be sold or offered for sale in California with a Clear and Reasonable Warning that complies with the provisions of this Section 3.2. A Clear and Reasonable Warning may only be provided for Covered Products that Settling Defendant reasonably believes do not meet the Reformulation Levels. A Clear and Reasonable Warning under this Agreement shall state:

WARNING: Consuming this product can expose you to chemicals including acrylamide, which are known to the State of California to cause cancer. For more information go to www.P65Warnings.ca.gov/food.

The word “**WARNING**” shall be displayed in all capital letters and bold print. This warning statement shall be prominently displayed on the Covered Product, on the packaging of the Covered Product, or on a placard or sign provided that the statement is displayed with such conspicuousness, as compared with other words, statements or designs as to render it likely to be read and understood by an ordinary individual prior to sale. If the warning statement is displayed on the Covered

Product's label, it must be set off from other surrounding information and enclosed in a text box. If the warning statement is displayed on a placard or sign where the Covered Product is offered for sale, the warning placard or sign must enable an ordinary individual to easily determine which specific Covered Products the warning applies to, and to differentiate between that Covered Product and other products to which the warning statement does not apply. For internet, catalog or any other sale where the consumer is not physically present, the warning statement shall be displayed in such a manner that it is likely to be read and understood by an ordinary individual prior to the authorization of or actual payment. Nothing in this Consent Judgment requires that warnings be provided for Covered Products that are not shipped for sale in California. If Settling Defendant elects to avail itself of the warning option provided by this Section 3.2, Settling Defendant shall provide written notice to CEH prior to Settling Defendant's first distribution or sale of Covered Products with warnings under this Section 3.2, and Settling Defendant concurrently shall make the additional payment specified in Section 5.2.4 below.

4. ENFORCEMENT

4.1 General Enforcement Provisions. CEH may, by motion or application for an order to show cause before this Court, enforce the terms and conditions contained in this Consent Judgment. Any action by CEH to enforce Settling Defendant's alleged violations of Section 3.1 or to enforce future alleged violations of Proposition 65 with respect to acrylamide exposures from the Covered Products shall be brought exclusively pursuant to this Section 4, and be subject to the meet and confer requirement of Section 4.2.4 if applicable.

4.2 Enforcement of Reformulation Commitment.

4.2.1 Notice of Violation. In the event that CEH purchases a Covered Product in California with a best-by or sell-by (or equivalent) date indicating that the Covered Product was sold or offered for sale by Settling Defendant after the Effective Date, and for which CEH has laboratory test results showing that the Covered Product exceeds the Unit Level, CEH may issue a Notice of Violation pursuant to this Section. CEH may not issue a Notice of Violation as to any Covered Product for which Settling Defendant has availed itself of the warning option under

1 Section 3.2 unless such Covered Product lacks a Clear and Reasonable Warning that complies
2 with Section 3.2

3 4.2.2 Service of Notice of Violation and Supporting Documentation.

4 4.2.2.1 The Notice of Violation shall be sent to the person(s) identified
5 in Section 8.2 to receive notices for Settling Defendant, and must be served within sixty (60) days
6 of the later of the date the Covered Product at issue was purchased by CEH or the date that CEH
7 can reasonably determine that the Covered Product at issue was manufactured, shipped, sold, or
8 offered for sale by Settling Defendant, provided, however, that CEH may have up to an additional
9 sixty (60) days to send the Notice of Violation if, notwithstanding CEH's good faith efforts, the
10 test data required by Section 4.2.2.2 below cannot be obtained by CEH from its laboratory before
11 expiration of the initial sixty (60) day period.

12 4.2.2.2 The Notice of Violation shall, at a minimum, set forth: (a) the
13 date the Covered Product was purchased; (b) the location at which the Covered Product was
14 purchased; (c) a description of the Covered Product giving rise to the alleged violation, including
15 the name and address of the retail entity from which the sample was obtained and pictures of the
16 product packaging from all sides, which identifies the product lot; and (d) all test data obtained by
17 CEH regarding the Covered Product and supporting documentation sufficient for validation of the
18 test results, including any laboratory reports, quality assurance reports, and quality control reports
19 associated with testing of the Covered Product.

20 4.2.3 Notice of Election of Response. No more than thirty (30) days after
21 effectuation of service of a Notice of Violation, Settling Defendant shall provide written notice to
22 CEH whether it elects to contest the allegations contained in a Notice of Violation ("Notice of
23 Election"). Failure to provide a Notice of Election within thirty (30) days of effectuation of
24 service of a Notice of Violation shall be deemed an election to contest the Notice of Violation.
25 Upon notice to CEH, Settling Defendant may have up to an additional sixty (60) days to elect if,
26 notwithstanding Settling Defendant's good faith efforts, Settling Defendant is unable to verify the
27 test data provided by CEH before expiration of the initial thirty (30) day period.

1 4.2.3.1 If a Notice of Violation is contested, the Notice of Election shall
 2 include all documents upon which Settling Defendant is relying to contest the alleged violation,
 3 including all available test data. If Settling Defendant or CEH later acquires additional test or
 4 other data regarding the alleged violation during the meet and confer period described in Section
 5 4.2.4, it shall notify the other Party and promptly provide all such data or information to the Party
 6 unless either the Notice of Violation or Notice of Election has been withdrawn.

7 4.2.4 Meet and Confer. If a Notice of Violation is contested, CEH and Settling
 8 Defendant shall meet and confer to attempt to resolve their dispute. Within thirty (30) days of
 9 serving a Notice of Election contesting a Notice of Violation, Settling Defendant may withdraw
 10 the original Notice of Election contesting the violation and serve a new Notice of Election to not
 11 contest the violation, provided, however, that, in this circumstance, Settling Defendant shall pay
 12 \$2,500 in addition to any other payment required under this Consent Judgment. At any time,
 13 CEH may withdraw a Notice of Violation, in which case for purposes of this Section 4.2 the
 14 result shall be as if CEH never issued any such Notice of Violation. If no informal resolution of a
 15 Notice of Violation results within thirty (30) days of a Notice of Election to contest, CEH may
 16 file an enforcement motion or application pursuant to Section 4.1. In any such proceeding, the
 17 prevailing party may seek whatever fines, costs, penalties, attorneys' fees, or other remedies are
 18 provided by law, including pursuant to Section 11, *infra*.

19 4.2.5 Non-Contested Notices. If Settling Defendant elects to not contest the
 20 allegations in a Notice of Violation, it shall undertake corrective action(s) and make payments, if
 21 any, as set forth below.

22 4.2.5.1 Settling Defendant shall include in its Notice of Election a
 23 detailed description with supporting documentation of the corrective action(s) that it has
 24 undertaken or proposes to undertake to address the alleged violation. Any such correction shall,
 25 at a minimum, provide reasonable assurance that all Covered Products having the same lot
 26 number as that of the Covered Product identified in CEH's Notice of Violation (the "Noticed
 27 Covered Products") will not be thereafter sold in California or offered for sale to California
 28

1 customers by Settling Defendant. Settling Defendant shall keep for a period of one year and
2 make available to CEH upon reasonable notice (which shall not exceed more than one request per
3 year) for inspection and copying records of any correspondence regarding the foregoing. If there
4 is a dispute over the corrective action, Settling Defendant and CEH shall meet and confer before
5 seeking any remedy in court. In no case shall CEH issue more than one Notice of Violation per
6 manufacturing lot of a type of Covered Product, nor shall CEH issue more than two Notices of
7 Violation in the first calendar year following the Effective Date.

8 4.2.5.2 If the Notice of Violation is the first, second, third, or fourth
9 Notice of Violation received by Settling Defendant under Section 4.2.1 that was not successfully
10 contested or withdrawn, then Settling Defendant shall pay \$15,000 for each Notice of Violation.
11 If Settling Defendant has received more than four (4) Notices of Violation under Section 4.2.1
12 that were not successfully contested or withdrawn, then Settling Defendant shall pay \$25,000 for
13 each Notice of Violation. If Settling Defendant produces with its Notice of Election test data for
14 the Covered Product that: (i) was conducted prior to the date CEH gave Notice of Violation;
15 (ii) was conducted on the same type of Covered Product; and (iii) demonstrates acrylamide levels
16 below the applicable Unit Level, then any payment under this Section shall be reduced by 100
17 percent (100%) for the first Notice of Violation, by seventy-five percent (75%) for the second
18 Notice of Violation, and by fifty percent (50%) for any subsequent Notice of Violation. In no
19 case shall Settling Defendant be obligated to pay more than \$100,000 for all Notices of Violation
20 not successfully contested or withdrawn in any calendar year irrespective of the total number of
21 Notices of Violation issued.

22 4.2.6 Payments. Any payments under Section 4.2 shall be made by check
23 payable to the “Lexington Law Group” and shall be paid within thirty (30) days of service of a
24 Notice of Election triggering a payment and shall be used as reimbursement for costs for
25 investigating, preparing, sending, and prosecuting Notices of Violation, and to reimburse
26 attorneys’ fees and costs incurred in connection with these activities.

1 **4.3 Repeat Violations.** If Settling Defendant has received four (4) or more Notices of
 2 Violation concerning the same type of Covered Product that were not successfully contested or
 3 withdrawn in any two (2) year period then, at CEH's option, CEH may seek whatever fines, costs,
 4 penalties, attorneys' fees, or other remedies that are provided by law for failure to comply with
 5 the Consent Judgment. Prior to seeking such relief, CEH shall meet and confer with Settling
 6 Defendant for at least thirty (30) days to determine if Settling Defendant and CEH can agree on
 7 measures that Settling Defendant can undertake to prevent future alleged violations.

8 **5. PAYMENTS**

9 **5.1 Payments by Settling Defendant.** Within twenty (20) calendar days of the
 10 Effective Date, Settling Defendant shall pay the total sum of \$80,000 as a settlement payment as
 11 further set forth in this Section.

12 **5.2 Allocation of Payments.** The total settlement amount shall be paid in four (4)
 13 separate checks in the amounts specified below and delivered as set forth below. Any failure by
 14 Settling Defendant to comply with the payment terms herein shall be subject to a stipulated late
 15 fee to be paid by Settling Defendant to CEH in the amount of \$100 for each day the full payment
 16 is not received after the payment due date set forth in Section 5.1. The late fees required under
 17 this Section shall be recoverable, together with reasonable attorneys' fees, in an enforcement
 18 proceeding brought pursuant to Section 4 of this Consent Judgment. The funds paid by Settling
 19 Defendant shall be allocated as set forth below between the following categories and made
 20 payable as follows:

21 **5.2.1** \$13,820 as a civil penalty pursuant to Health & Safety Code § 25249.7(b).
 22 The civil penalty payment shall be apportioned in accordance with Health & Safety Code §
 23 25249.12 (25% to CEH and 75% to the State of California's Office of Environmental Health
 24 Hazard Assessment ("OEHHA")). Accordingly, the OEHHA portion of the civil penalty
 25 payment for \$10,365 shall be made payable to OEHHA and associated with taxpayer
 26 identification number 68-0284486. This payment shall be delivered as follows:
 27
 28

1 For United States Postal Service Delivery:

2 Attn: Mike Gyurics
3 Fiscal Operations Branch Chief
4 Office of Environmental Health Hazard Assessment
5 P.O. Box 4010, MS #19B
6 Sacramento, CA 95812-4010

7 For Non-United States Postal Service Delivery:

8 Attn: Mike Gyurics
9 Fiscal Operations Branch Chief
10 Office of Environmental Health Hazard Assessment
11 1001 I Street, MS #19B
12 Sacramento, CA 95814

13 The CEH portion of the civil penalty payment for \$3,455 shall be made payable to
14 the Center for Environmental Health and associated with taxpayer identification number 94-
15 3251981. This payment shall be delivered to Lexington Law Group, 503 Divisadero Street, San
16 Francisco, CA 94117.

17 5.2.2 \$10,360 as an Additional Settlement Payment (“ASP”) to CEH pursuant to
18 Health & Safety Code § 25249.7(b), and California Code of Regulations, Title 11, § 3204. CEH
19 intends to restrict use of the ASPs received from this Consent Judgment to the following
20 purposes: the funds will be placed in CEH’s Toxics in Food Fund and used to support CEH
21 programs and activities that seek to educate the public about acrylamide and other toxic
22 chemicals in food, to work with the food industry and agriculture interests to reduce exposure to
23 acrylamide and other toxic chemicals in food, and to thereby reduce the public health impacts and
24 risks of exposure to acrylamide and other toxic chemicals in food sold in California. CEH shall
25 obtain and maintain adequate records to document that ASPs are spent on these activities and
26 CEH agrees to provide such documentation to the Attorney General within thirty (30) days of any
27 request from the Attorney General. The payment pursuant to this Section shall be made payable
28 to the Center for Environmental Health and associated with taxpayer identification number 94-
3251981. This payment shall be delivered to Lexington Law Group, 503 Divisadero Street, San
Francisco, CA 94117.

1 5.2.3 \$55,820 as a reimbursement of a portion of CEH’s reasonable attorneys’
 2 fees and costs. The attorneys’ fees and cost reimbursement shall be made payable to the
 3 Lexington Law Group and associated with taxpayer identification number 94-3317175. This
 4 payment shall be delivered to Lexington Law Group, 503 Divisadero Street, San Francisco, CA
 5 94117.

6 5.2.4 **Additional Civil Penalty.** If Settling Defendant avails itself of the
 7 warning option provided for by Section 3.2, Settling Defendant shall make an additional
 8 payment of \$80,000 as a civil penalty, concurrently with its written notice as provided in
 9 Section 3.2. This additional civil penalty payment shall be apportioned in accordance with
 10 Health & Safety Code § 25249.12 (25% to CEH and 75% to the State of California’s Office of
 11 Environmental Health Hazard Assessment (“OEHHA”). Accordingly, the OEHHA portion of
 12 the civil penalty payment for \$60,000 shall be made payable to OEHHA, associated with
 13 taxpayer identification number 68-0284486, and sent to the OEHHA address set forth in section
 14 5.2.1 above or any updated address for OEHHA. The CEH portion of the additional civil
 15 penalty payment for \$20,000 shall be made payable to the Center for Environmental Health and
 16 associated with taxpayer identification number 94-3251981. This payment shall be delivered to
 17 Lexington Law Group, 503 Divisadero Street, San Francisco, CA 94117.

18 **6. MODIFICATION AND DISPUTE RESOLUTION**

19 6.1 **Modification.** This Consent Judgment may be modified from time to time by
 20 express written agreement of the Parties, with the approval of the Court and prior notice to the
 21 Attorney General’s Office, or by an order of this Court upon motion and prior notice to the
 22 Attorney General’s Office and in accordance with law.

23 6.2 **Notice; Meet and Confer.** Any Party seeking to modify this Consent Judgment
 24 shall attempt in good faith to meet and confer with the other Party prior to filing a motion to
 25 modify the Consent Judgment.

26 6.3 In the event that new legislation or regulations relating to the acrylamide content
 27 of the Covered Products is adopted on either the federal or California state level, after meeting
 28

1 and conferring pursuant to Section 6.2 above, either Party may seek a modification to conform the
2 requirements of this Consent Judgment to such new requirements provided that the requirements
3 are either: (a) at least as restrictive as those set forth herein; or (b) completely preemptive of
4 Proposition 65 as adjudged by a final order of an appellate court of competent jurisdiction, and
5 the other Party may oppose such a modification.

6 **7. CLAIMS COVERED AND RELEASE**

7 7.1 This Consent Judgment is a full, final and binding resolution between CEH, on
8 behalf of itself and the public interest, and Settling Defendant and its parents, subsidiaries,
9 affiliated entities that are under common ownership, directors, officers, employees, agents,
10 shareholders, successors, assigns, and attorneys (“Defendant Releasees”), and all entities to which
11 Settling Defendant directly or indirectly distribute or sell Covered Products, including but not
12 limited to distributors, wholesalers, customers, retailers, franchisees, licensors, and licensees
13 (including without limitation Bristol Farms, Inc.) (“Downstream Defendant Releasees”), of any
14 violation of Proposition 65 based on failure to warn about alleged exposure to acrylamide
15 contained in Covered Products that were sold, distributed, or offered for sale by Settling
16 Defendant prior to the Effective Date.

17 7.2 In consideration of Settling Defendant’s obligations under Section 5, CEH, for
18 itself, its agents, successors and assigns, releases, waives, and forever discharges any and all
19 claims against Settling Defendant, Defendant Releasees, and Downstream Defendant Releasees
20 arising from any violation of Proposition 65 or any other statutory or common law claims that
21 have been or could have been asserted by CEH individually or in the public interest regarding the
22 failure to warn about exposure to acrylamide arising in connection with Covered Products
23 manufactured, distributed or sold by Settling Defendant prior to the Effective Date.

24 7.3 Compliance with the terms of this Consent Judgment by Settling Defendant shall
25 constitute compliance with Proposition 65 by Settling Defendant, Defendant Releasees and
26 Downstream Defendant Releasees with respect to any alleged failure to warn about acrylamide in
27 Covered Products manufactured, distributed, or sold by Settling Defendant after the Effective
28

1 Date.

2 **8. PROVISION OF NOTICE**

3 8.1 When CEH is entitled to receive any notice under this Consent Judgment, the
4 notice shall be sent by first class and electronic mail to:

5 Howard Hirsch
6 Lexington Law Group
7 503 Divisadero Street
8 San Francisco, CA 94117
9 hhirsch@lexlawgroup.com

10 8.2 When Settling Defendant is entitled to receive any notice under this Consent
11 Judgment, the notice shall be sent by first class and electronic mail to:

12 George Gigounas
13 DLA Piper LLP
14 555 Mission Street, Suite 2400
15 San Francisco, CA 94105
16 George.Gigounas@dlapiper.com

17 Richard Fama
18 Cozen O'Connor
19 45 Broadway Atrium, Suite 1600
20 New York, NY 10006
21 rfama@cozen.com

22 Any Party may modify the person and/or address to whom the notice is to be sent by sending
23 the other Party notice by first class and electronic mail.

24 **9. COURT APPROVAL**

25 9.1 This Consent Judgment shall become effective upon the date signed by CEH and
26 Settling Defendant, whichever is later, provided however, that CEH shall prepare and file a
27 Motion for Approval of this Consent Judgment and Settling Defendant shall support entry of this
28 Consent Judgment by the Court.

9.2 If this Consent Judgment is not entered by the Court, it shall be of no force or
effect and shall not be introduced into evidence or otherwise used in any proceeding for any
purpose other than to allow the Court to determine if there was a material breach of Section 9.1.

1 **10. GOVERNING LAW AND CONSTRUCTION**

2 10.1 The terms of this Consent Judgment shall be governed by the laws of the State of
3 California.

4 **11. ATTORNEYS' FEES**

5 11.1 A Party who unsuccessfully brings or contests an action, motion, or application
6 arising out of this Consent Judgment shall be required to pay the prevailing Party's reasonable
7 attorneys' fees and costs.

8 11.2 Nothing in this Section 11 shall preclude a party from seeking an award of
9 sanctions pursuant to law.

10 **12. ENTIRE AGREEMENT**

11 12.1 This Consent Judgment contains the sole and entire agreement and understanding
12 of the Parties with respect to the entire subject matter hereof, and any and all prior discussions,
13 negotiations, commitments, or understandings related thereto, if any, are hereby merged herein
14 and therein. There are no warranties, representations, or other agreements between the Parties
15 except as expressly set forth herein. No representations, oral or otherwise, express or implied,
16 other than those specifically referred to in this Consent Judgment have been made by any Party
17 hereto. No other agreements not specifically contained or referenced herein, oral or otherwise,
18 shall be deemed to exist or to bind any of the Parties hereto. Any agreements specifically
19 contained or referenced herein, oral or otherwise, shall be deemed to exist or to bind any of the
20 Parties hereto only to the extent that they are expressly incorporated herein. No supplementation,
21 modification, waiver, or termination of this Consent Judgment shall be binding unless executed in
22 writing by the Party to be bound thereby. No waiver of any of the provisions of this Consent
23 Judgment shall be deemed or shall constitute a waiver of any of the other provisions hereof
24 whether or not similar, nor shall such waiver constitute a continuing waiver.

25 **13. RETENTION OF JURISDICTION**

26 13.1 This Court shall retain jurisdiction of this matter to implement or modify the
27 Consent Judgment.

1 **14. AUTHORITY TO STIPULATE TO CONSENT JUDGMENT**

2 14.1 Each signatory to this Consent Judgment certifies that he or she is fully authorized
3 by the Party he or she represents to stipulate to this Consent Judgment and to enter into and
4 execute the Consent Judgment on behalf of the Party represented and legally to bind that Party.

5 **15. OTHER SETTLEMENTS**

6 15.1 Nothing in this Consent Judgment shall preclude CEH from resolving any claim
7 against any other entity on terms that are different from those contained in this Consent
8 Judgment.

9 15.2 Settling Defendant may move to modify this Consent Judgment pursuant to
10 Section 6 to substitute higher Reformulation Levels that CEH agrees to in a future consent
11 judgment applicable to products identical to the Covered Products, and CEH agrees not to oppose
12 any such motion except for good cause shown.

13 **16. CHANGE IN LAW**

14 16.1 In the event that Proposition 65 is repealed, preempted, or is otherwise rendered
15 inapplicable by reason of law generally, or if any of the provisions of this Consent Judgment are
16 rendered inapplicable or are no longer required as a result of any such repeal or preemption, or
17 rendered inapplicable by reason of law generally as to the Covered Products, then Defendant may
18 provide written notice to Plaintiff of any asserted change in the law, and shall have no further
19 obligations pursuant to this Consent Judgment with respect to, and to the extent that, the Covered
20 Products are so affected.

21 16.2 Nothing in this Consent Judgment shall be interpreted to relieve Defendant from
22 any obligation to comply with any other pertinent state or federal law or regulation.

17. EXECUTION IN COUNTERPARTS

17.1 The stipulations to this Consent Judgment may be executed in counterparts and by means of facsimile or portable document format (pdf), which taken together shall be deemed to constitute one document.

IT IS SO ORDERED, ADJUDGED, AND
DECREED.

Dated: DEC 18 2018

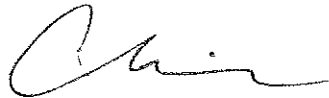
IOANA PETROU

Judge of the Superior Court

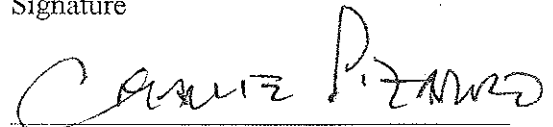
IT IS SO STIPULATED:

Dated: 18 Oct, 2018

CENTER FOR ENVIRONMENTAL HEALTH



Signature



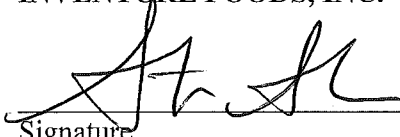
Printed Name



Title

1 Dated: 10/12, 2018
2
3

INVENTURE FOODS, INC.


Signature

4
5 Steven Sklar
6 Printed Name

7 SVP & GM
8 Title
9

EXHIBIT 1

Boulder Canyon Sweet Potato Chips

Boulder Canyon Sweet Potato Fries

Boulder Canyon Sweet Potato Skins

Nathan's Crinkle Cut Fries – all flavors

TGIF Sweet Potato Skins

Vidalia Sweet Potato Fries

Exhibit 2

Exhibit A

COVERED PRODUCTS

CORN, GRAIN, AND LEGUME CHIPS AND STICKS

Group A. All corn, grain, and legume-based chips and sticks manufactured by Settling Defendant, including El Sabroso Guacachips, El Sabroso Jalapenitos, Private Label Tortilla Chips, Private Label Organic Blue Tortilla Chips, Private Label Organic Fiesta Tortilla Chips, Private Label Organic White Tortilla Chips, Whole Earth Really Seedy Tortilla Chips, El Sabroso Reduced Fat Tortilla Chips, Private Label Reduced Fat Tortilla Chips, Granny Goose Restaurant Style Tortilla Chips, Private Label Organic Yellow Rounds Tortilla Chips, El Sabroso Salsitas, El Sabroso Yellow Rounds Tortilla Chips, Granny Goose White Corn Tortilla Strips, Private Label White Corn Tortilla Strips, El Sabroso Chile Y Limon Churritos, El Sabroso Chile Y Limon Corn Chips, Granny Goose Corn Chips

Type 1: Triangle-shaped chips

Type 2: Round, rolled, and other non-triangle or non-strip-shaped chips

Type 3: Strip-shaped chips

Type 4: Corn chips and corn sticks (e.g., churritos)

POPCORN

Group B. All popcorn products, including Snak King Popcorn (Cheddar Cheese and Butter), Granny Goose Butter Popcorn, Kettle Corn, Whole Earth Lightly Salted Popcorn, Private Label Organic Popcorn (White Cheddar and Light Salt), Granny Goose Caramel Popcorn

Type 1: Popcorn (plain, flavored and kettle)

Type 2: Caramel and candy corn (with or without nuts)

EXTRUDED, PELLET, AND BAKED PRODUCTS

Group C. All extruded, pellet, and baked products (excluding baked products in Group A), including Private Label Lavash Chips, Private Label Salted Pita Chips, Whole Earth Salted Pita Chips, Private Label Hot Fries, Snak King Hot Fries, Private Label Puffed Rice or Corn, Snak King Cheese Puffs, Private Label Cheese Puffs, Private Label Rice Balls, Private Label Multigrain Chips, Private

Label Baked Cheese Curls, Granny Goose Cheese Blazin Curls, Snak King Baked Cheese Curls, Snak King Fried Cheese Curls, Snak King Hot Cheese Curls, Jensen Orchards Veggie Chips, Private Label Veggie Sticks, Private Label Mini Veggie Chips, El Sabroso Duros, Private Label Popped Chips

Type 1: Pita and lavash chips (all flavors)

Type 2: Puffs, fries, baked curls, and multigrain chips (all flavors)

Type 3: Fried curls (all flavors)

Type 4: Potato, vegetable, and other grain-based pellet chips and sticks (all flavors)

Type 5: Duros (all flavors)

PRETZELS

Group D. All pretzels

Type 1: Twists and sticks

OTHER

Group E. All pork rinds and “cracklins,” including El Sabroso Regular Pork Rinds, El Sabroso Regular Pork Rinds with Salsa, El Sabroso Hot & Spicy Pork Rinds, El Sabroso Regular Cracklins, and El Sabroso Hot & Spicy Cracklins.

Type 1: Pork rinds and “cracklins”

EXHIBIT 12

GLICK LAW GROUP, P.C.

Noam Glick (SBN 251582)
225 Broadway, Suite 2100
San Diego, California 92101
Tel: (619) 382-3400
Fax: (619) 615-2193
Email: noam@glicklawgroup.com

NICHOLAS & TOMASEVIC, LLP

Craig M. Nicholas (SBN 178444)
Shaun Markley (SBN 291785)
Jake Schulte (SBN 293777)
225 Broadway, 19th Floor
San Diego, California 92101
Tel: (619) 325-0492
Fax: (619) 325-0496
Email: cnicholas@nicholaslaw.org
Email: smarkley@nicholaslaw.org
Email: jschulte@nicholaslaw.org

Attorneys for Plaintiff
Kim Embry

**SUPERIOR COURT OF THE STATE OF CALIFORNIA
IN AND FOR THE COUNTY OF ALAMEDA**

KIM EMBRY, an individual

Plaintiff,

v.

NONNI'S FOODS, LLC., a Delaware
corporation, WALMART, INC., a Delaware
corporation, and DOES 1 through 100,
inclusive

Defendants.

Case No. HG-17-885297

[PROPOSED] CONSENT JUDGMENT AS
TO NONNI'S FOODS, LLC.

1 **1. INTRODUCTION**

2 **1.1 Parties**

3 This Consent Judgment is entered into by and between Kim Embry (“Embry”) and Nonni’s
4 Foods, LLC (“Defendant or “Nonni’s”) (collectively the “Parties”).

5 **1.2 Plaintiff**

6 Embry is an individual residing in California and acting in the interest of the general public.
7 She seeks to promote awareness of exposures to toxic chemicals and to improve human health by
8 reducing or eliminating hazardous substances in consumer products.

9 **1.3 Defendant**

10 Nonni’s employs ten or more individuals and is a “person in the course of doing business” for
11 purposes of the Safe Drinking Water and Toxic Enforcement Act of 1986, Health and Safety Code
12 section 25249.6 et seq. (“Proposition 65”).

13 **1.4 General Allegations**

14 Embry alleges that Nonni’s manufactures, imports, sells, and distributes for sale baked biscotti
15 (“Italian cookie”) products that contain acrylamide. Embry further alleges that Nonni’s does so
16 without providing a sufficient health hazard warning as required by Proposition 65 and related
17 Regulations. Pursuant to Proposition 65, acrylamide is listed as a chemical known to cause cancer and
18 reproductive harm.

19 **1.5 Covered Products**

20 For purposes of this Consent Judgment “Covered Products” means all baked biscotti (“Italian
21 cookie”) products containing acrylamide, including but not limited to traditional biscotti,
22 THINaddictives and biscotti cookie bites, that are manufactured, imported, sold, or distributed by
23 Defendant Releasees, defined below, for sale in California.

24 **1.6 Releasees**

25 “Releasees” means and includes: A. Nonni’s, its parents, subsidiaries, affiliated entities,
26 directors, officers, employees, agents, shareholders, successors, assigns, insurers, and attorneys (the
27 “Defendant Releasees”) and all entities to which Defendant Releasees directly or indirectly distribute
28 or sell Covered Products, including but not limited to distributors, wholesalers, customers, retailers,

1 franchisees, licensors, and licensees, including but not limited to Walmart Inc. its parents, subsidiaries,
2 affiliated entities, directors, officers, employees, agents, shareholders, successors, assigns, insurers,
3 and attorneys (the "Downstream Defendant Releasees").

4 **1.7 Notices of Violation**

5 On March 15, 2019 Embry served Defendant, the California Attorney General, and all other
6 required public enforcement agencies with a 60-Day Notice of Violation of California Health and
7 Safety Code section 25249.6 *et seq.* ("Notice"). The Notice alleged that Defendant violated
8 Proposition 65 by failing to sufficiently warn consumers in California of the health hazards associated
9 with exposures to Acrylamide contained in the Products.

10 No public enforcer has commenced or is otherwise prosecuting an action to enforce the
11 violations alleged in the Notice.

12 **1.8 Complaint**

13 On or about _____, Embry filed a Complaint against Defendant alleging the
14 violations of Health and Safety Code section 25249.6 that are the subject of the Notice ("Complaint").

15 **1.9 No Admission**

16 Defendant denies the material factual and legal allegations of the Notice and Complaint, and
17 maintains that all of the products it has manufactured, imported, sold, and/or distributed for sale in
18 California, including Covered Products, have been, and are, in compliance with all laws. Nothing in
19 this Consent Judgment shall be construed as an admission of any fact, finding, conclusion of law, issue
20 of law, or violation of law, nor shall compliance with this Consent Judgment be construed as an
21 admission of any fact, finding, conclusion of law, issue of law, or violation of law. This Section shall
22 not, however, diminish or otherwise affect Defendant's obligations, responsibilities, and duties under
23 this Consent Judgment.

24 **1.10 Jurisdiction**

25 For purposes of this Consent Judgment and the Complaint only, the Parties stipulate that this
26 Court has jurisdiction over Defendant as to the allegations in the Complaint, that venue is proper in
27 the County of Alameda, and that the Court has jurisdiction to enter and enforce the provisions of this
28 Consent Judgment pursuant to Proposition 65 and Code of Civil Procedure section 664.6.

1 **1.11 Effective Date**

2 For purposes of this Consent Judgment, the term “Effective Date” means that date certain
3 falling six calendar months after the date on which the Court grants the motion for approval and entry
4 of this Consent Judgment, as discussed in Section 5.

5 **2. INJUNCTIVE RELIEF**

6 **2.1 Reformulation of the Product**

7 Commencing on the Effective Date, and continuing thereafter, Defendant Releasees shall only
8 manufacture, ship, sell, or offer for sale Covered Products that: (a) contain an average acrylamide
9 concentration by weight (the “Average Level”) of 280 parts per billion or less; or (b) are labeled with
10 a clear and reasonable warning pursuant to Section 2.2. The Average Level shall be determined: (a)
11 by randomly selecting and testing at least one sample each from five different lots of the product (or
12 the maximum number of lots available for testing if less than five) that were produced on dates spread
13 out roughly evenly over a period of at least 60 days; and (b) using tests performed by a laboratory
14 accredited by the State of California, a federal agency, or a nationally recognized accrediting
15 organization, using LC-MS (Liquid Chromatograph-Mass Spectrometry) or any other testing method
16 agreed upon by the Parties.

17 **2.2 Clear and Reasonable Warnings**

18 Commencing on the Effective Date and continuing thereafter, Defendant Releasees shall, for
19 all Covered Products that do not contain an Average Level of 280 parts per billion or less, provide
20 clear and reasonable warnings as set forth in Proposition 65 and related Regulations.

21 In the event that the Office of Environmental Health Hazard Assessment promulgates one or
22 more regulations requiring or permitting warning text, permitting the absence of warning text, and/or
23 permitting methods of transmission different than those set forth above, Defendant Releasees shall be
24 entitled to use, at their discretion, such other warning text and/or method of transmission without being
25 deemed in breach of this Consent Judgment.

26 **2.3 Sell-Through Period**

27 Notwithstanding anything else in this Settlement Agreement, Covered Products that were
28 manufactured before the Effective Date shall be subject to a full release of all liability pursuant to this

1 Consent Judgment, without regard to when such Covered Products were, or are in the future,
 2 distributed or sold to customers. The obligations of Defendant Releasees, do not apply to Covered
 3 Products manufactured before the Effective Date. Claims concerning those earlier manufactured
 4 products are released nonetheless.

5 **2.4 Court Approval of Less Onerous Compliance Measures**

6 If a California court approves a Proposition 65 consent judgment concerning acrylamide for
 7 one or more competitors of any Defendant Releasee that provides for materially less onerous
 8 compliance measures, the Court, upon application by Nonni's, shall modify this Consent Judgment to
 9 replace the more onerous compliance measures set forth herein with those less onerous compliance
 10 measures.

11 **3. MONETARY SETTLEMENT TERMS**

12 **3.1 Settlement Amount**

13 Nonni's shall pay sixty-five thousand dollars (\$65,000) in settlement and total satisfaction of
 14 all the claims referred to in the Notice, the Complaint, and this Consent Judgment. This includes civil
 15 penalties in the amount of seven thousand dollars (\$7,000) pursuant to Health and Safety Code section
 16 25249.7(b) and attorney's fees and costs in the amount of fifty-eight thousand dollars (\$58,000)
 17 pursuant to Code of Civil Procedure section 1021.5.

18 **3.2 Civil Penalty**

19 The portion of the settlement attributable to civil penalties shall be allocated according to
 20 Health and Safety Code section 25249.12(c)(1) and (d), with seventy-five percent (75%) of the penalty
 21 paid to the California Office of Environmental Health Hazard Assessment ("OEHHHA"), and the
 22 remaining twenty-five percent (25%) of the penalty paid to Embry individually.

23 All payments owed to Embry shall be delivered to the following payment addresses:

24 Noam Glick
 25 Glick Law Group
 26 225 Broadway, Suite 2100
 San Diego, CA 92101

27 All payments owed to OEHHHA (EIN: 68-0284486) shall be delivered directly to EOHHA (Memo
 28 line "Prop 65 Penalties) at the following addresses:

For United States Postal Delivery:

Mike Gyuries
Fiscal Operations Branch Chief
Office of Environmental Health Hazard Assessment
P.O Box 4010
Sacramento, CA 95812-4010

For Non-United States Postal Service Delivery:

Mike Gyuries
Fiscal Operations Branch Chief
Office of Environmental Health Hazard Assessment
1001 I Street
Sacramento, CA 95814

Nonni's agrees to provide Embry's counsel with a copy of the check payable to OEHHA, simultaneous with its penalty payments to Embry.

The Parties, including Embry, will exchange completed IRS 1099, W-9, or other forms as required. Relevant information for Glick Law Group, N&T, and Embry are set out below:

- "Kim Embry" whose address and tax identification number shall be provided within five (5) days after this Consent Judgement is fully executed by the Parties
- "Glick Law Group" (EIN: 47-1838518) at address provided in Section 3.2;
- "Nicholas & Tomasevic" (EIN: 46-3474065) at address provided in Section 3.3; and
- "Office of Environmental Health Hazard Assessment at 1001 I Street, Sacramento, CA 95814.

3.3 Attorney's Fees and Costs

The portion of the settlement attributable to attorney's fees and costs fifty-eight thousand dollars (\$58,000) shall be paid to Embry's counsel, who are entitled to attorney's fees and costs incurred by her in this action, including but not limited to investigating potential violations, bringing this matter to Nonni's attention, as well as litigating and negotiating a settlement in the public interest.

1 Nonni's shall provide its payment to Embry's counsel in two checks, divided equally, payable
2 to Glick Law Group, PC twenty-nine thousand dollars (\$29,000) and Nicholas & Tomasevic, LLP
3 twenty-nine thousand dollars (\$29,000) respectively. The addresses for these two entities are:

4
5 Noam Glick
6 Glick Law Group
7 225 Broadway, Suite 2100
8 San Diego, CA 92101

9 Crag Nicholas
10 Nicholas & Tomasevic, LLP
11 225 Broadway, 19th Floor
12 San Diego, CA 92101

13 **3.4 Timing**

14 The above-mentioned checks will be issued within fourteen (14) days of the Effective Date.

15 **4. CLAIMS COVERED AND RELEASED**

16 **4.1 Embry's Public Release of Proposition 65 Claims**

17 Embry, acting for the general public, releases each and all Releasees from all claims arising
18 under Proposition 65, based on exposure to and/or failure to warn about exposure to, acrylamide from
19 Covered Products manufactured, imported, sold, or distributed before the Effective Date.

20 Compliance with the terms of this Consent Judgment constitutes compliance with Proposition
21 65 with respect to all alleged or actual failure(s) to warn about exposures to acrylamide from Covered
22 Products that are manufactured, imported, sold, or distributed after the Effective Date. This Consent
23 Judgment is a full, final and binding resolution of all claims that were or could have been asserted
24 against Releasees for exposure to acrylamide from Covered Products and/or failure to warn about
25 exposure to acrylamide from Covered Products.

26 **4.2 Embry's Individual Release of Claims**

27 Embry, in her individual capacity, hereby releases each and all Releasees, which shall be a full
28 and final accord and satisfaction of, as well as a bar to, all actions, causes of action, obligations, costs,
expenses, attorney's fees, damages, losses, claims, liabilities, and demands of every nature, character,
and kind, whether known or unknown, suspected or unsuspected, arising out of alleged or actual

1 exposures to acrylamide in Covered Products manufactured, imported, sold, or distributed by
2 Releasees before the Effective Date, and/or that were or could have been alleged or asserted in the
3 Complaint.

4 **4.3 Defendant's Release of Embry**

5 Nonni's, for itself and the Defendant Releasees, hereby waives any and all claims against
6 Embry and her attorneys and other representatives, for any and all actions taken or statements made
7 by Embry and her attorneys and other representatives, whether in the course of investigating claims or
8 otherwise, committed or omitted in the process of seeking to enforce Proposition 65 against it with
9 respect to Covered Products through the date of Nonni's execution of this Stipulation.

10 **5. COURT APPROVAL**

11 This Consent Judgment is not effective until it is approved and entered by the Court and shall
12 be null and void if it is not approved and entered by the Court within one year after it has been fully
13 executed by the Parties, or by such additional time as the Parties may agree to in writing.

14 **6. SEVERABILITY**

15 Subsequent to the Court's approval and entry of this Consent Judgment, if any provision is
16 held by a court to be unenforceable, the validity of the remaining provisions shall not be adversely
17 affected.

18 **7. GOVERNING LAW**

19 The terms of this Consent Judgment shall be governed by the laws of the state of California as
20 applied within the state. If Proposition 65 is repealed, or is otherwise rendered inapplicable for any
21 reason, including but not limited to changes in the law, then Nonni's shall have no further obligations
22 pursuant to this Consent Judgment with respect to, and to the extent that, the Covered Products are so
23 affected.

24 **8. NOTICE**

25 Unless otherwise specified herein, all correspondence and notice required or permitted by this
26 Consent Judgment shall be in writing and sent: (1) by personal delivery or by US Mail (first-class,
27 registered, or certified mail, return receipt requested), or by a recognized overnight courier, to the
28

physical address provided below, (2) with copies, not themselves constituting notice, emailed to each email address provided below:

If to Nonni's:

Lucas Quass
Latham & Watkins, LLP
650 Town Center Drive, 20th Floor
Costa Mesa, CA 92626

If to Embry:

Noam Glick
Glick Law Group, P.C.
225 Broadway, 21st Floor
San Diego, CA 92101

Any Party may, from time to time, specify in writing to the other, a change of mailing or email addresses to which notices and other communications shall be sent. Any and all Notices shall be effective only if sent in compliance with this section, and after the emailed copies have been sent without reported error.

9. COUNTERPARTS; DIGITAL SIGNATURES

This Consent Judgment may be executed in counterparts and executed by digital, faxed, or otherwise reproduced signature. Each counterpart shall be deemed an original, and all counterparts taken together shall constitute one and the same document.

10. POST EXECUTION ACTIVITIES

Embry agrees to comply with the reporting form requirements referenced in Health and Safety Code section 25249.7(f). The Parties further acknowledge that, pursuant to Health and Safety Code section 25249.7(f), a noticed motion is required to obtain judicial approval of this proposed settlement, which motion Embry shall draft and file. In furtherance of obtaining such approval, the Parties agree to mutually employ commercially reasonable efforts, including those of their counsel, to support the entry of this agreement as judgment, and to obtain judicial approval of their settlement in a timely manner. For purposes of this Section, "commercially reasonable efforts" shall include, at a minimum, supporting the motion for approval, responding to any objection that any third-party may make, and appearing at the hearing before the Court if so requested.

11. MODIFICATION

This Consent Judgment may be modified by: (i) a written agreement of the Parties and entry of a modified consent judgment thereon by the Court; or (ii) a successful motion or application of any Party, and the entry of a modified consent judgment thereon by the Court.

1 **12. AUTHORIZATION**

2 The undersigned are authorized to execute this Consent Judgment and acknowledge that they
3 have read, understand, and agree to all of the terms and conditions contained herein.

4 **13. GOOD FAITH ATTEMPT TO RESOLVE DISPUTES**


5 If a dispute arises with respect to either Party's compliance with the terms of this Consent
6 Judgment entered by the Court, the Parties shall meet and confer in person, by telephone, and/or in
7 writing and endeavor to resolve the dispute in an amicable manner. No action or motion may be filed
8 in the absence of such a good faith attempt to resolve the dispute beforehand.

9 **14. ENTIRE AGREEMENT**

10 This Consent Judgment contains the sole and entire agreement and understanding of the Parties
11 with respect to the entire subject matter herein, and any and all prior discussions, negotiations,
12 commitments, and understandings related hereto. No representations, oral or otherwise, express or
13 implied, other than those contained herein have been made by any Party. No other agreements, oral or
14 otherwise, unless specifically referred to herein, shall be deemed to exist or to bind any Party.

15
16
17 **AGREED TO:**

18 Date: September 25, 2019

19 By: 
20 KIM EMBRY

AGREED TO BY (DEFENDANT)

Date: 9/25/2019


By: 
Brian T Hansberry [print name]

EXHIBIT 13

K PRIME, INC.
LABORATORY REPORT

SAMPLE ID: DEVIL'S FOOD COOKIE CAKES -
NEW RECIPE - SAMPLE 1

LAB NO: 186769

DATE SAMPLED: NA

TIME SAMPLED: NA

BATCH #: 082819P2

DATE EXTRACTED: 08/28/2019

DATE ANALYZED: 08/30/2019

K PRIME PROJECT: [REDACTED]
CLIENT PROJECT: B&G FOODS

METHOD: ACRYLAMIDE IN FOOD
REFERENCE: ID-GC/MS/MS

SAMPLE TYPE: PRODUCT
UNITS: ug/Kg (PPB)

COMPOUND NAME	CAS NO.	REPORTING LIMIT	SAMPLE RESULT
ACRYLAMIDE	79-06-1	40.0	47.6

NOTES:

ND - NOT DETECTED AT OR ABOVE THE STATED REPORTING LIMIT

NA - NOT APPLICABLE OR AVAILABLE

APPROVED BY: AB
DATE: 9/3/19

K PRIME, INC.
LABORATORY REPORT**SAMPLE ID:** DEVIL'S FOOD COOKIE CAKES -
NEW RECIPE - SAMPLE 2**LAB NO:** 186770**DATE SAMPLED:** NA**TIME SAMPLED:** NA**BATCH #:** 082819P1**DATE EXTRACTED:** 08/28/2019**DATE ANALYZED:** 08/30/2019**K PRIME PROJECT:** [REDACTED]
CLIENT PROJECT: B&G FOODS**METHOD:** ACRYLAMIDE IN FOOD
REFERENCE: ID-GC/MS/MS**SAMPLE TYPE:** PRODUCT
UNITS: ug/Kg (PPB)

COMPOUND NAME	CAS NO.	REPORTING LIMIT	SAMPLE RESULT
ACRYLAMIDE	79-06-1	40.0	65.2

NOTES:

ND - NOT DETECTED AT OR ABOVE THE STATED REPORTING LIMIT

NA - NOT APPLICABLE OR AVAILABLE

APPROVED BY: AB
DATE: 9/3/19

K PRIME, INC.
LABORATORY REPORTSAMPLE ID: DEVIL'S FOOD COOKIE CAKES -
NEW RECIPE - SAMPLE 3

LAB NO: 186771

DATE SAMPLED: NA

TIME SAMPLED: NA

BATCH #: 082819P2

DATE EXTRACTED: 08/28/2019

DATE ANALYZED: 08/30/2019

K PRIME PROJECT: XXXXXXXXXX
CLIENT PROJECT: B&G FOODSMETHOD: ACRYLAMIDE IN FOOD
REFERENCE: ID-GC/MS/MSSAMPLE TYPE: PRODUCT
UNITS: ug/Kg (PPB)

COMPOUND NAME	CAS NO.	REPORTING LIMIT	SAMPLE RESULT
ACRYLAMIDE	79-06-1	40.0	73.1

NOTES:

ND - NOT DETECTED AT OR ABOVE THE STATED REPORTING LIMIT

NA - NOT APPLICABLE OR AVAILABLE

APPROVED BY: AB
DATE: 9/3/19